VOL. V MARCH, 1918 No. 3

SOIL SCIENCE

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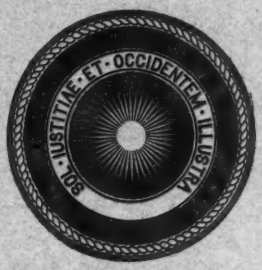

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PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter August 23, 1917, at the post office at Baltimore, Maryland, under the act of March 3, 1879.
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SOIL SCIENCE

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SOIL ACIDITY: I. ITS RELATION TO THE GROWTH OF PLANTS

EMIL TRUOG

Department of Soils, Agricultural Experiment Station, University of Wisconsin

Received for publication February 15, 1918

It has been known for a long time that certain species of plants are affected unfavorably by soil acidity or what is sometimes called a lack of lime, while in this respect other species are indifferent or may even be affected favorably by an acid soil condition or one of low lime supply. On this basis Contejean (5) made a botanical classification of plants under three general heads: viz., the calciphile, or lime-loving, the calcifuge, or lime-avoiding, and the indifferent. While this system of classification may be valuable from a botanical standpoint, it is not adapted for use in a detailed consideration of the relation of soil acidity and lime to our common agricultural plants, for with a few exceptions all of these plants grow best on soils well supplied with lime. This point is further emphasized by Hilgard (22) who has frequently called attention to the adage,—"A limestone country is a rich country," and also the statement of Hall (17) that any soil containing less than 1 per cent of calcium carbonate will be benefited by liming. The beneficial effects of liming soils on agricultural plants are often due partly to indirect actions of the lime, in which case the use of the term calciphile, or lime-loving, might carry the wrong impression as to the mode of action of the lime.

In this connection it is interesting to note that recent experiments by Pipal (33) of the Indiana Station and White (47) of the Pennsylvania Station show that sorrel (*Rumex Acetosella*) which is commonly supposed to be a lime-avoiding plant is really benefited by liming. As they and also Frear (7) and others have indicated, sorrel is usually found growing on very acid soils because there it meets with the least competition from other weeds and also field crops. It would be found growing even better on limed soils were it not that here other more vigorous plants grow so well that they crowd out the sorrel. Undoubtedly, competition working in conjunction with soil acidity or alkalinity in the way just indicated has a marked influence on the powers of different species of plants for establishing and maintaining them-

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station. This is the first number of a series of articles on the subject of soil acidity. These investigations were made possible through the support and encouragement of Professor A. R. Whitson, chairman of the Department of Soils. The writer is indebted to Professors H. W. Ullsperger, W. W. Weir and L. F. Graber for suggestions regarding practical aspects of this subject.

selves under natural conditions (18) and thus has affected the character of the native vegetation found in many regions.

Another method of classification of plants, especially the economic ones, which has been used to some extent in this connection is the division into acid-tolerant, acid-intolerant and indifferent. This method of classification has some desirable features as well as some undesirable ones. It is liable to carry the impression that the acidity of the soil actually attacks the tissues of the plant roots, which, as will be indicated later, is undoubtedly seldom the case. Moreover, it tends to minimize or detract from the importance of factors which operate in an indirect way, and which are sometimes the controlling ones.

The subject of the relation of soil acidity to the growth of plants is really so complex that it seems almost impossible to get a simple system of classification of economic agricultural plants that may be adapted for general use in this connection. The detailed consideration of the harmful effects of soil acidity which follows will bring this out more clearly and forcibly. A considerable number of reasons have been suggested or put forward at various times as to the way in which soil acidity injuriously affects the growth of plants. These may be classified under the following heads:

A. The indirect and general influence of soil acidity on plant growth due to its effect on:
   1. The general fertility of the soil.
   2. The prevalence of plant diseases.
   3. The competitive powers of different species of plants.

B. The direct and specific influence of soil acidity on plant growth due to its effect on:
   1. The supply of available calcium needed by plants as direct plant-food material.
   2. The symbiotic nitrogen-fixing bacteria of the legumes.
   3. The root tissues of plants.

A. THE INDIRECT INFLUENCE OF SOIL ACIDITY ON PLANT GROWTH

1. Effect on the general fertility

It is generally recognized that soil acidity has marked unfavorable influences on the general fertility of soils. These unfavorable influences are due to the effects of soil acidity on the physical, biological and chemical conditions and processes of soils.

The physical condition of clay soils, especially, is generally known to be affected unfavorably by an acid condition, due to an insufficient supply of calcium bicarbonate in the soil solution to keep the clay particles flocculated (16), which condition is necessary for the highly desirable granular or crumb structure. In sandy soils the presence of lime carbonate probably improves the physical condition by acting as a binding agent. In improving the physical condition of soils in these ways, the presence of lime carbonate thus indirectly helps in the proper regulation of the air and moisture supply of soils, which, in turn, is the fundamental basis for developing the proper biological and chemical conditions of soils.

It is fully recognized at present that for nearly all crops an acid soil condition is unfavorable to the highest development of the desirable biological conditions and processes of soils. Nitrification and all forms of nitrogen fixation are often markedly checked by an acid condition. Soil acidity by checking these processes acts as a preservative of the organic matter of soils, and thus prevents or delays the liberation of the plant-food elements bound up in the organic matter itself, as well as the elements in the mineral matter which would be made available by the action of the products of decomposing organic matter. Toxic organic substances may also be preserved (43) and thus tend to accumulate to a harmful degree in acid soils much more than in the non-acid ones.

By direct chemical reactions, as Sykora and the writer (43) have shown, lime carbonate may precipitate and thus inhibit the action of substances toxic to plants. In fact nearly all the chemical reactions which take place in soils are affected unfavorably in regard to fertility by an acid condition.

Under an acid soil condition, the soil phosphorus in particular eventually appears to become less available (38) as a result principally of two reasons: viz., (a)the gradual conversion of the soil phosphorus from calcium phosphate into iron and aluminum phosphates, in which condition the phosphorus becomes with time less and less soluble (42) in the soil solution; and (b) the formation of physical and perhaps chemical complexes of the phosphates with the acidic organic matter (31), in which condition the phosphorus is of low availability because of the very slow rate of decomposition, under acid conditions, of the organic matter, which serves as a protective layer physically and chemically. The addition of lime sometimes retards the immediate effectiveness of insoluble phosphate fertilizers (42), but this is undoubtedly only a temporary condition.[2]

Not only the phosphorus but practically all the soil elements are probably affected as to solubility and availability by the reaction of the soil, whether acid or alkaline. From a purely chemical standpoint it would seem that some elements, namely, calcium, magnesium, sodium and potassium would become less available when the soil becomes acid, while a second group of elements, namely, iron, aluminum, manganese, copper, zinc and other heavy metals, would become more soluble and available. The elements of the first group, especially calcium, constitute the great regulators of the reaction of the soil solution. There are three main reasons why the availability of the first group of elements is usually lowered when a soil becomes acid: viz., (a) the supply of these elements in the form of various compounds becomes less as soil acidity develops; (b) the soil solution must compete for these elements with the insoluble soil acids causing soil acidity; and (c) the possibili-

[2] The addition of lime to acid soils sometimes retards the growth of immediately following crops which have low lime needs, like oats, wheat and corn. This is due to temporary conditions which soon disappear and the lime then greatly benefits these crops as well as those with high lime needs.

ties of solution of these elements are lessened by a lowering of the biological activities which results from soil acidity.

A lowering in availability and concentration in the soil solution of the first-named group of elements in the form of carbonates, especially calcium, is favorable to an increase in solubility and concentration in the soil solution of the second-named group of elements. This is due to the fact that the carbonates of the first group, especially calcium, act as precipitating agents for the elements of the second-named group which might otherwise be in solution in the form of various soluble salts. Soil acidity is thus favorable to a high solubility in the soil solution of the second-named group of elements.

Soil acidity may thus in some cases indirectly affect fertility by limiting the availability of the potassium. Besides neutralizing soil acids which otherwise compete with the soil solution for potassium, the addition of lime may possible in some cases, as has been often assumed, displace potassium in insoluble compounds, and thus make it more available (30), although the recent investigations of Briggs and Breazeale (4) and also others do not support this assumption.

The addition of lime may also make the magnesium more available in the same ways as potassium. This addition of lime to acid soils may in some special cases, where the percentage of magnesia much exceeds that of lime, result in a more favorable lime-magnesia ratio. It is to be noted, however, that an acid soil condition probably lowers the solubility of the magnesia in the soil solution much more than of the calcium oxide, because of the much greater affinity of magnesia (27) for the insoluble soil acids. This is further substantiated by the fact that in most cases dolomitic limestone gives good results when used on acid soils.

With respect to availability or solubility, sodium is affected in practically the same way as potassium, but as regards fertility this is of little importance.

Of the group of elements whose concentration in the soil solution is decreased (29) by the addition of lime to acid soils, iron is one of the most important. This decrease is desirable in that it lessens the chances for the formation of iron phosphate. However, on the other hand, in certain cases an excess of lime may lower the concentration of iron in the soil solution, or the permeability of the root hairs, so much that certain plants like the pineapple (13, 14, 24) and certain varieties of the lupine (32) actually suffer from a lack of iron and become chlorotic, although other plants are not seriously affected unfavorably.

Considerable amounts of manganese have been noted by the writer in the soil solution of certain acid soils. When lime was added to these soils the manganese was not found in any considerable amounts in the soil solution because of the fact that an alkaline condition is favorable for its precipitation. As the writer (42) has previously indicated, this high manganese content of the soil solution of acid soils may possibly in some cases act injuriously on certain plants, because of a high absorption of this element. This idea is

supported by the investigations of White (47) which show that the addition of lime to acid soils lowers the manganese content of clover and sorrel grown thereon. The investigations of Wilcox, Kelly (48) and Johnson (24) emphasize the importance of these manganese relations.

An acid condition is also favorable for the formation of soluble aluminum salts (29) which are believed to be toxic (34) to plants. Hartwell and Pember (20) place considerable emphasis on this indirect effect of soil acidity. The investigations of the Indiana Station (1) on an acid, peaty soil indicated that aluminum nitrate was present in sufficient amounts in the soil solution of this soil to be decidedly toxic to plants. The addition of lime resulted in a precipitation of the aluminum and a prevention of the toxic action. The use of ammonium sulfate without lime may result in the formation of considerable aluminum sulfate which is toxic to plants. Cases of soil acidity in which soluble aluminum salts accumulate in seriously toxic amounts are, however, probably quite exceptional. Acid peaty and sandy soils and others that have received considerable ammonium sulfate or other sulfates and chlorides are most liable to develop a toxic condition in this respect as a result of the solubility of aluminum under these conditions. Silt loams and clays, because of the high "buffer"[3] effects of their large amounts of colloidal matter, undoubtedly largely overcome toxic influences of this kind.

Copper, zinc and other heavy metals if present in the soil in appreciable amounts, either naturally or as a result of their addition in various ways, may become soluble and toxic especially under acid conditions.

As has been shown by Sykora and the writer (43) the addition of lime prevents the toxic action of soluble copper salts. This is probably effected by precipitation of the copper as the insoluble carbonate. Undoubtedly the toxic effects in acid soils of zinc, lead and some others of the heavy metals may be prevented by liming in the same way as with copper. This relation of acidity and lime to possible toxic effects of strong mineral acids and heavy metals is probably a point of considerable importance in certain mining regions where smelter fumes or other waste materials are brought to the soil or where soils have received considerable amounts of certain fertilizers, soil amendments or spray materials.

2. Effect on prevalence of plant diseases

In this connection it is important to note that certain fungous diseases like "finger-and-toe" develop to a harmful extent only in acid soils, while other plant diseases like the potato scab are most serious in recently limed soils, and hence at least slight acidity is desirable under many conditions for potato growing. Aside from the cases known to have a direct relation to

[3] The usual meaning of the term "buffer" is given in a footnote page 177. It is used here in a general sense as applying not only to H-ion concentration, but to the concentration of all the constituents in the soil solution.

plant diseases, there are a few special plants like the cranberry, blackberry and water-melon (19) for which a soil of at least slight acidity seems desirable and in some cases necessary for the best growth. It is not known just why these plants grow better on an acid soil, but it seems possible that in some cases plant diseases or mal-nutrition due to a lack of iron where lime is added may again be factors.

3. Effect on plant competition

The influence of soil acidity or alkalinity on the competitive powers of different species of plants for establishing themselves and crowding out others has already been discussed and its method of operation explained. Under native conditions or in case land is left in meadow or pasture for a considerable number of years (18) this influence may be a factor of considerable importance. However, under most farming conditions, which are the primary concern of this paper, this form of competition is largely eliminated by cultivation, and hence need not receive further consideration in this discussion.

General considerations

While it is thus possible to find a few (comparatively very few) cultivated plants that for direct or indirect reasons actually grow better on acid soils than on neutral or alkaline ones, yet it is most important to note that nearly all of our important agricultural plants grow best on neutral or alkaline soils. Reasons have been given why the general fertility of soils is affected favorably by the use of lime on acid soils, but in perhaps 95 per cent of the actual cases of soil acidity there is a vastly more important specific influence on certain plants and there always remains the important question,—why are some of the important agricultural crops affected very much more than others by an acid condition and benefited so much more than the others by the use of lime? For example, why is alfalfa affected more by an acid condition than is oats, or even medium red clover, and why are sugar beets affected much more than potatoes? Apparently these very important differences are not fully explained by the considerations already given regarding the general fertility of the soil, plant competition or plant diseases. In order, therefore, to explain these differences a number of possible *direct* and *specific* influences of soil acidity as already outlined have been suggested at various times. These will now be considered.

B. THE DIRECT AND SPECIFIC INFLUENCE OF SOIL ACIDITY

1. Effect on availability of calcium

The influence of soil acidity on the supply of available calcium which is needed by plants as direct plant-food material has been suggested to explain

at least in part the question under consideration. Brief reference to this has already been made under the head of general fertility, but because of the special *direct* relations of certain plants it is now taken up again and considered more fully. As has been explained, when soils become acid the supply of available calcium in all forms becomes less. That the supply of calcium as plant-food material is not often the limiting factor in acid soils is shown by the fact that the addition of calcium sulfate (19) does not supply the deficiency of these acid soils. Calcium enters the tissues of plants as a necessary constitutent or integral part to only a very small extent. Large amounts are found, however, in many plants in combination with organic acids which are probably by-products of vital life processes. Only calcium in the form of the carbonate or in forms that pass over to the carbonate in the soil meet this special large requirement of certain plants, and hence the calcium as the carbonate seems to be needed directly by these plants for other uses than as plant-food material. These other uses are discussed in detail beginning on page 179.

2. Effect on symbiotic nitrogen-fixing bacteria

Since some of the principal legumes are usually greatly benefited by the liming of acid soils, the statement is commonly made that these legumes are sensitive to acidity or are acid intolerant because the nitrogen-fixing bacteria of these legumes are unable to do their work satisfactorily under acid soil conditions. The fact that certain non-legumes are just as injuriously affected by acid soil conditions as certain legumes is evidence that the action of these acid conditions need not necessarily be directly on the legume bacteria which live in the nodules.

In regard to this matter Wheeler (46) has pointed out the fallacy of the common notion in the following words: "On account of a lack of sufficient appreciation of these conditions, the agricultural press and even scientific publications often contain statements to the effect that legumes are in great need of liming, in order that they may develop root nodules and properly assimilate atmospheric nitrogen. Nevertheless the Southern cowpea, serradella, and certain of the lupines are likely to be injured by heavy liming."

Since the legume bacteria live actively in the nodules, the normal medium of these bacteria would seem to be one which is acid to a certain degree, inasmuch as the nodules are in direct connection with the rest of the plant whose circulatory sap is known to be acid. Contrary to the common statement that these legume bacteria require an alkaline medium for active development, it thus appears that they thrive in a medium which is acid to a certain degree, and as the data of Fred and Graul (8) show, even the alfalfa bacteria fix nitrogen to a considerable extent in some acid soils.

There is, however, a time when an acid condition of the soil directly affects the legume bacteria and that is while they are still in the soil prior to the

symbiotic relationship with the plant. The writer has noticed that in case a soil is quite acid, the acidity seems to prevent or delay the plants in becoming infected with the bacteria even though the soil has been thoroughly inoculated with the proper bacteria. Fred and Loomis (10) have shown that when the acidity of media reaches a certain degree the multiplication of alfalfa bacteria is greatly lessened, and hence it is reasonable to believe that an acid condition of the soil affects the acivity, multiplication, rate of movement and hence distribution of the legume bacteria in the soil itself in such a way that the chances for infection of the plant are greatly lessened. This may be due partially to the effects of an acid condition in retarding the neutralization and decomposition of acidic and toxic products, and on the nature and supply of available phosphates (39) and other food material. Phosphates (9) especially are known to have a decided effect on bacterial activity. The supply of calcium carbonate and bicarbonate in the soil solution is generally known to affect greatly the activity of the soil bacteria. This supply is directly affected by an acid condition of the soil.

Frequently in pot cultures of alfalfa on acid soils it happens that at first only one or a few plants become infected even though the soil had been thoroughly inoculated. These plants that become infected grow much more rapidly and become much darker in color than the uninfected ones indicating that if the legume bacteria once gain entrance to the plant, they are able to fix nitrogen fairly energetically even though the soil is quite acid.

The specific influence of soil acidity on the growth of plants thus does not appear to be due to a direct action on the symbiotic nitrogen-fixing bacteria in the nodules.

3. Effect on the root tissues of plants

The injurious influence of soil acidity on the growth of certain plants is sometimes assumed to be due to the toxic or destructive effect of the soil acids on the root tissues of the plants. The following statement taken from a well known textbook (26) of soils illustrates this point:

> Free acids are toxic to most agricultural plants. Some plants are much more sensitive than others. Alfalfa, for example, should have a slightly alkaline medium for its best growth, and any acid is very injurious.

Why an acid should be more toxic or destructive to alfalfa roots than to clover roots, or to barley roots than to oats roots, is certainly neither easily explained nor plausible from a chemical or physiological standpoint. Since the sap of practically all plants is acid, and often more acid than the soil solution of the common acid soils, the fallacy of the contention that soil acids often directly affect the root tissues at once becomes apparent. The consideration which immediately follows brings this out clearly.

In an investigation of the acidity[4] (H-ion concentration) of the plant sap of a number of agricultural plants, M. R. Meacham[5] and the writer found that this varies at least between the H-ion exponents of 4.0 and 6.0, or in other terms between the acidities represented by 1/10,000 N and 1/1,000,000 N solutions. The addition of lime to acid soils usually caused a slight lowering in the acidities of the saps of plants grown on these soils. Determination by N. E. Loomis[5] and the writer of the H-ion concentrations of soil extracts from a considerable number of the common cultivated acid soils of Wisconsin of varying degrees of acidity gave a range of H-ion exponents of 4.5 to 8.0. Similarly, in an investigation of a considerable number of acid and alkaline soils, Gillespie (15) found a range of H-ion exponents of 4.4 to 8.6, and Sharp and Hoagland (35) a range of 3.7 to 9.7. It is thus apparent that the acidity of the plant saps is of the same order as that of the water extracts of acid soils, and hence it is not to be expected that the acidity of the soil solution often becomes high enough to be directly seriously toxic or destructive to the plant roots.

The similar and in most cases restricted range of H-ion concentrations of plant saps and soil solutions indicates that similar processes are probably at work in the two cases because of certain analogous conditions which exercise a regulatory function in this respect. Since proteins are *amphoteric*[6] and exert a tremendous *buffer*[7] effect on solutions which bathe them, it is readily explained why the sap of most plants has a rather restricted range of H-ion concentrations. Similarly, the soil constituents, especially the colloidal

[4] The reader is reminded that acidity expressed in terms of H-ion concentration refers only to what is sometimes called "true" acidity resulting from the ionized part of the acids. In many chemical processes and especially in biological acitivity, it has been shown that the effects of acidity are usually due largely to the ionized acidity of H-ions. This does not, however, preclude marked effects from un-ionized acidity in certain cases. The reader is also reminded that Sorensen's (37) method of expressing H-ion concentrations by means of H-ion exponents which is used here is interpreted as follows:

The exponent expressed is the negative exponent or power to which 10 must be raised in order to give the H-ion concentration in grams per liter. Thus:—

Exponent 1.0 means 10^{-1} or $\frac{1}{10}$ gm. H-ions per liter or $\frac{1}{10}$ N acid.

Exponent 5.0 means 10^{-5} or $\frac{1}{100,000}$ gm. H-ions per liter or $\frac{1}{100,000}$ N acid.

An exponent of 7.0 which is that of pure water designates neutrality while one greater than 7.0 designates alkalinity, and one less than 7.0 acidity. The character P_H. proposed by Sorensen is sometimes used to designate the expression—H-ion exponent, or power.

[5] A detailed account of this work will be given in the succeeding numbers of this series of articles on Soil Acidity.

[6] The term "amphoteric" applies to substances which like the proteins may act as either acids or bases to indicators or the surrounding solution. Colloidal soil constituents like organic matter, acid silicates, kaolin, phosphates, iron and aluminum salts and oxides and many others are amphoteric to the soil solution. As the soil solution tends to become more acid these amphoteric substances prevent any rapid change by combining with the acid and also by giving up base to the solution.

[7] The term "buffer" refers to substances which tend to preserve the original H-ion concentration. Salts in solution as well as colloidal matter may act as buffers.

organic and inorganic matter, exert a tremendous buffer effect on the H-ion concentration of the soil solution and in this way restrict and regulate this concentration or acidity to such an extent that the soil solution is usually not acid enough to be directly toxic or destructive to plant root tissues. As the writer (41) has previously pointed out the action of other plant toxins is also inhibited in this way.

The work of Hartwell and Pember (21) of the Rhode Island Station indicates that an acidity of 1/5000 N, HCl, or less, which it is to be noted is more acid than the water extracts of most, very strongly acid soils, has no apparent injury on oats, rye, wheat and barley seedlings. These investigators also observed that although barley gains more than rye through the addition of lime to acid soils, yet it is not more sensitive directly to acidity, although it does appear to be to aluminum (20) salts.

Gedroitz (12) also made investigations along this line with water cultures and found the following order of acid resistance beginning with the least resistant: flax, mustard, vetch and barley. Thus barley, although less sensitive directly to acidity, usually responds more to liming than flax, again indicating that the so-called acid intolerance is not due to a direct action of the soil acids on the plants. The acidities which the different plants withstood without injury varied from 1/1000 N to 1/5000 N solutions.

In carefully controlled experiments, Hoagland (23) recently found that a culture having an acidity expressed by an H-ion exponent of 5.2 is very favorable for the growth of barley, and he states that it would appear from these experiments that acid soils with an H-ion concentration of 0.8×10^{-5} (H-ion exponent of 5.1) or less would not be injurious to plants of the cereal group because of excessive acidity.

In certain *unusual* cases of very strongly acid soils, especially peats and sands, it is possible that the acidity of the soil solution may be high enough to affect the plant directly to a serious extent. The writer has on several occasions noted a depression in growth following the addition of soluble fertilizer salts to an unlimed, strongly acid soil. The addition of a salt like K_2SO_4 to an acid soil results, of course, in an increased acidity of the soil solution due to the partial removal of the base by the insoluble soil acids and the liberation of an equivalent amount of free sulfuric acid, or acid sulfate, which being soluble, exerts a much stronger action on the plant than the original insoluble soil acids, and may also increase the soluble aluminum or other salts to a toxic concentration.

It should be noted in this connection that the soil solution in contact with growing plant roots is continually drawn on for nitrates, phosphates and sulfates and receives in return carbonic acid. This not only lowers the possible concentration of soluble mineral acids in the immediate region of the absorbing roots, but the carbonic acid, together with that liberated from decomposing organic matter and biological life, acts on the soil silicates and forms a certain amount of carbonates which undoubtedly have a regulatory influence

on the acidity of the soil solution, especially in the immediate region of the feeding roots, in the same way as they are known to have in other mediums. That this carbonic acid really acts to a considerable extent as a regulator of the acidity of the soil solution of acid soils is indicated by the fact that when even very strongly acid mineral soils are extracted with carbonated water, there is obtained an extract which on boiling is alkaline to phenolphthalein.

General considerations

In most of the common cases of soil acidity it thus appears that the specific harmful influence of the acidity on certain plants is usually not due in any large degree to any of the three suggested reasons which have just been discussed. What appears to the writer to be the most probable way in which this specific influence is exerted will now be discussed and explained in detail.

THE LIME REQUIREMENTS OF PLANTS

Definitions

The designation "lime requirement of soils" has been a common method of speaking of the apparent amount of acidity in soils, but as will be indicated, it is much better to speak of the degree of acidity of soils or acidity directly in some appropriate terms, and use the expression "lime requirement" in reference to plants and not to soils.

The expression "lime requirement of a soil" has usually been understood to mean the amount of lime needed to neutralize the total acidity of the soil, and it has also been quite generally believed that enough lime should be used in practice to neutralize all of this acidity. As the writer (44) has previously indicated, the total acidity of a soil may be very great—much greater than has generally been supposed—and yet in practice it is only necessary to neutralize a small fraction of the total acidity. The amount of lime that can be most profitably used on an acid soil depends not only on the degree of acidity but also on the crop to be grown and the fertility of the soil. Thus the expression "lime requirement of an acid soil" must either have reference to the total acidity of the soil, or if it is to have reference to the amount of lime which should be applied, then, for any particular soil, it is a varying quantity depending on the crop to be grown. As is readily seen, this method of expression leads to a great deal of confusion. Fortunately, this confusion can easily be avoided by adopting a simple method of expressing the degree of acidity of a soil and using the expression "lime requirement" in reference to plants instead of soils.

As the writer (42) has previously indicated, it is usually because the actual lime needs of a plant are not fulfilled that soil acidity exerts a specific injurious influence on certain plants, and not because the acidity is directly toxic or

destructive. Lime is needed by plants for at least two purposes: viz., (1) to furnish the comparatively small amounts of calcium needed as an indispensable constituent of plant substances, in which case it is regarded as a plant-food material; and (2) to act as a neutralizing and precipitating agent of the acids in the plant sap, which are formed as by-products of vital phenomena. Undoubtedly calcium salts of different kinds are suitable for furnishing the calcium needed for the first purpose, but only the carbonate or bicarbonate are suitable for the second purpose. It is undoubtedly for this second purpose that a large part of the calcium found in plants is used, especially in the case of plants which require exceptionally large amounts of calcium.

The expression "lime requirement of a plant" has been used in a general way, but because of insufficient knowledge regarding the relation of soil acidity and liming to plants it has not been clearly defined. It has often been used in referring in a general way to the relation of plant growth to the lime supply of the soil. In order to avoid confusion as to what is meant in the discussions that follow, the writer gives the following definition: *The expression "lime requirement of a plant" refers to the actual lime needs of the plant itself, especially as to the ease and rate at which lime must be secured from the soil by the plant for normal growth.* Thus if a plant has a high lime requirement, then the solution and delivery must be easy and rapid in order to meet the needs of the plant. If a plant has a low lime requirement, then the rate of solution and delivery need also be only low. As is easily seen the rate at which lime must be secured is determined by the lime content and rate of growth of the plant, and the ease with which it must be secured is determined by the feeding power of the plant for lime. The factors which determine the lime requirement may thus be outlined as follows:

1. Lime content of the plant.
2. Rate of growth of the plant.
3. Feeding power of the plant for lime.
   a. Extent of root system.
   b. Character of root system.
   c. Internal acidity of roots.
   d. Excretion of carbonic acid.

It is to be clearly noted that not only the lime content but also the rate of growth and feeding power of a plant are factors that determine the lime requirement of a plant. The feeding power of a plant for lime depends largely on the extent and character of the root system. As is well known, the oat plant has a much more extensive root system than the barley plant and hence is also a much more vigorous feeder. Plants with fibrous and branching root systems that extend and spread fully through the cultivated layer usually have a much stronger feeding power than the tap-rooted plants. Many varieties of alfalfa are tap rooted, and as is well known their rather low feed-

ing power compared with their rate of growth is reflected in the high fertility required for their successful growth. The clovers and some varieties of alfalfa, like Grimm, have a more profusely branched root system which enables them to be grown successfully on poorer and more acid soil. The deep tap-rooted plants, of course, withstand drought better.

Another point in this connection which should be clearly noted is the difference in absorbing or feeding powers of new and old roots. It is at or near the growing regions of the roots where most of the feeding takes place, and hence a plant, of which the cowpea is an excellent example, which grows rapidly and soon produces a fair root system, has a strong feeding power. A comparatively new and hence very active root system of this kind may have a stronger feeding power than a more extensive but older, hardened and less active system. It is probably for this reason that the cowpea is a much stronger feeder than medium red clover. The ability of a plant to grow rapidly on a poor soil is good evidence of a strong feeding power, and conversely, if a plant requires a very rich soil a weak feeding power is indicated.

The investigations of Meacham and the writer previously referred to show that the internal acidity of some plants is considerably higher than of others. Buckwheat, which has a very high feeding power, as is shown by its ability to grow on poor soils, has a very high internal acidity which probably accounts at least partly for this high feeding power in spite of the rather restricted root system. A high internal acidity undoubtedly makes possible a membrane which is more permeable to calcium that is presented as the bicarbonate and also expedites the progressive passage of the calcium salt within the plant. The internal acidity of a plant may thus affect the feeding power even though appreciable amounts of the acids other than carbonic are not excreted by the roots. Carbonic acid is, as is well known, excreted in large amounts by all plant roots.

If these principles regarding the feeding powers of plants are now applied, it becomes evident that if a plant has a high lime content, grows rapidly and has a rather low or medium feeding power, then its lime requirement is very high. Of this, the alfalfa plant is an exceptionally good example. If a plant has a fairly high lime content, grows rapidly, but an exceptionally strong feeding power, then its lime requirement is only medium or low, as is the case with the cowpea and buckwheat. If a plant has a low lime content, a rapid rate of growth but an exceptionally strong feeding power, then its lime requirement is very low, as is the case with oats.

The relation of soil acidity to the lime requirement of plants

As has already been indicated, when mineral soils, even the strongly acid ones, are extracted with carbonated water there are obtained extracts which after boiling are alkaline to phenolphthalein, indicating that in the natural soil solution of even strongly acid mineral soils there is dissolved calcium

bicarbonate, especially in the region of active plant roots where considerable amounts of carbonic acid are produced. Acting on this suggestion, E. Hay[8] and the writer have devised a comparative method for extracting calcium from acid soils with carbonated water. Determinations were made with this method on a considerable number of acid soils and the results correlated with the growths that alfalfa made on these same soils when fertilized but not limed. This correlation revealed a marked parallelism between the growth of the alfalfa and the calcium that was extracted. This indicates that the specific injurious influence of soil acidity on alfalfa and perhaps certain other plants is due to the lack of sufficient calcium as the bicarbonate in the soil solution. In a previous publication the writer (42) pointed this out and explained the mechanism of the reactions involved as follows:

> When not disturbed, the soil solution comes to a state of equilibrium with the phosphates, silicates, carbonates, organic compounds and other solid compounds. In case a soil is acid, then solid carbonates in appreciable amounts are usually absent. Hydrolysis and carbonation are the principal processes that bring dissolved mineral substances into the soil solution.
>
> The equilibrium conditions between the carbonic acid in the soil solution and the solid insoluble soil acids and soil silicates may be taken to illustrate the point under discussion. The insoluble soil acids of the acid soil may be represented by H_2X, and $CaSiO_3$ may be taken as a representative silicate. In the soil there are probably present acid silicates which in this system may replace either the soil acids or silicate. In this system of soil acids silicate and carbonic acid, the following reactions are possible.
>
> (1) $CaSiO_3 + 2H_2CO_3 \rightleftarrows H_2SiO_3 + CaH_2(CO_3)_2$
> (2) $CaSiO_3 + H_2X \rightleftarrows H_2SiO_3 + CaX$
> (3) $CaH_2(CO_3)_2 + H_2X \rightleftarrows 2H_2CO_3 + CaX$
>
> As is evident from these reactions, the concentration of calcium bicarbonate in solution at equilibrium will depend upon, besides the concentration of carbonic acid and temperature, the amount of surface exposed by the calcium silicate and especially upon the amount and *strength* of soil acids present causing soil acidity. If considerable amounts of relatively strong soil acids are present then the concentration and rate of formation and solution of calcium bicarbonate and delivery to the plant will be too low to meet the maximum need of growing alfalfa and certain other plants and hence the growths of these plants will be checked.

The writer has found that the strength, or avidity, of the active soil acids, as well as the amount, is a very important factor in affecting the amount of calcium bicarbonate extracted with carbonated water. The test which the writer (40) has devised for soil acidity gives a collective result called *the degree of acidity* which embodies a measure of both the strength and amount of active acids present in a soil. It is thus especially well adapted in the practical testing of soils.

It is thus evident that the insoluble soil acids are a direct factor in the possibility of a soil meeting the lime requirement of a particular species of plant.

[8] A detailed account of this method and investigation will be given in one of the succeeding numbers of this series of articles on soil acidity.

The relation of soil fertility to the lime requirements of plants

There are many soils in Wisconsin which are medium to strongly acid and yet they are quite fertile. These soils grow fairly good crops of corn, oats, wheat and medium red clover. Some even grow fair crops of alfalfa for at least one or two years, but when such soils are classified it is found that they invariably fall into one of two groups: viz., either (a) they are comparatively new soils which are well supplied witn minerals containing the essential elements, or else (b) they are soils which have been heavily fertilized with farm manure. It is to be noted that silicious sandy soils are never found in the first group, and particularly that all soils of at least slight to medium acidity, which have been farmed in a general way for twenty-five years or more with little use of manure, invariably require the addition of lime for the successful production of alfalfa, and often even of red clover. The theory which supposes that the specific effect of soil acidity on plants and bacteria is a toxic or destructive one falls down entirely in explaining this situation, since it does not explain why the same degree of acidity is more injurious in one soil than in another. If the theory which is based on the lime requirements of plants is used, the situation is easily and satisfactorily explained as follows:

It is first important to note that the soils of the two groups which have just been discussed always either have a good supply of calcium silicates and compounds, or they are very active chemically and biologically in the processes that bring calcium bicarbonate into the soil solution, or else they are soils in which there exists a combination of these two conditions. When these soils are extracted with carbonated water, large amounts of calcium as bicarbonate are brought into solution and reference to the mechanism of the reactions involved which have just been described makes it possible to explain why such should be the case. Another point to be emphasized is that acid soils, which are fertile otherwise, quickly produce plants of sufficient vigor to exert their own maximum feeding power for the comparatively insoluble lime compounds. It is now apparent that the reason clover and sometimes even alfalfa grow well on acid soils of the two groups just described is that the plants' lime requirements are satisfied by these soils in spite of the acidity.

The relation of the lime requirements of plants to their degrees of response to liming acid soils

If the theory which is proposed is a tenable one then it should be possible to show that there is a close parallel relation between the lime requirements of plants and their degrees of response to the liming of acid soils, or reciprocally to their ability to grow on acid soils. Unfortunately, there are no data at hand from experiments which were deliberately designed for critically testing this relationship. The data of the Rhode Island Station (19) resulting from extensive investigations by Wheeler, Hartwell and their associates,

are by far the most comprehensive of any dealing with this subject and hence these data have been used a great deal in estimating the relative degrees of response of plants to liming as given in the last column of table 1.

There may be some objections to the use of the Rhode Island data for the present general purpose, because of the considerable amounts of commercial fertilizers which were annually used, and which are known to have a considerable influence on the direct and indirect effects which soil acidity has on plant growth. In the case of the plots which received ammonium sulfate but no lime, the acidity was so abnormally strong that many plants made practically no growth, and hence it is difficult to make reliable comparisons in these cases as to the influence of soil acidity. The other set of unlimed plots, having received sodium nitrate and acid phosphate, are also not especially well adapted for comparison as unlimed plots, since the use of sodium nitrate lowers the acidity and acid phosphate furnishes calcium which under these conditions may be delivered to the plant as calcium carbonate or bicarbonate, and quite largely supply a plant's need in this respect. Because of such reasons as have been given, these data in some cases do not bring out differences which are known to exist from results of other experiments as well as practical farm observations. In the case of the clovers, for example, differences in the response to liming are not indicated, while it seems to be generally believed that alsike will still make a fairly good growth when medium red clover fails because of soil acidity. Undoubtedly plant diseases are sometimes partially the cause of differences in this case. Similarly, crimson clover is also known to grow under more acid conditions than medium red.

In using these data of the Rhode Island Station the aim has been to strike a general average of all the data pertaining to any particular species of plant. In arriving at the final figure for designating the degree of response to liming or the reciprocal figure for ability to grow on acid soils, other scattered available data as well as the writer's own plant house and field observations have been considered and incorporated. The writer therefore regards these figures as being tentative until checked up by experiments which are deliberately planned to test out these points critically.

In arriving at the figures which designate the lime requirements of plants, uncertainties have also arisen in some cases, largely because of the lack of specific data regarding the feeding power of plants for lime. On pages 180 and 181 the factors which influence this feeding power are discussed.

As is indicated in the table the data on the calcium oxide content of plants has been taken quite largely from Wolff's "Aschen Analysen." Wherever possible an attempt was made to secure averages of a considerable number of analyses. This, however, was not always possible, partly because of the desirability of selecting analyses which were made from material cut in the green condition, and in case of a group of plants like the clovers, which are to be critically compared with one another, the necessity of using analyses made on plants which have reached the same stage of maturity. In the

case of alfalfa, the clovers and some other plants, isolated analyses may be found which, relatively, are quite different from those given. This is especially true in case the plants had reached maturity or were cut as hay. It has been conclusively shown that plants may lose a large amount of salts (25), especially lime salts, when allowed to ripen or to be leached after being cut as hay.

Where close comparisons are to be made between similar plants of a group, it is also desirable that the analyses represent plants grown on the same kind of soil and under the same climatic conditions. Because of the great importance of the clovers and alfalfa, attention is called to the fact that the average analyses given for these plants are relatively the same as those reported by Way and Ogston (45), which were made on plants grown under similar conditions.

It is also important to note that the lime content of only the green aerial parts of plants is considered, since it is in these parts that lime is largely needed as an indispensable material for vital phenomena, and hence they give a much better indication of the lime needs of a plant than storage roots, tubers, and woody parts like the trunks of trees.

The relative rate of plant growth refers to the rate of production of green aerial parts in dry weight. In many cases the figures given are simply estimates, which, however can usually be made with considerable confidence.

In the table, abbreviated designations are used as follows:

1 designates – very low
2 designates – low
3 designates – medium
4 designates – high
5 designates – very high

Intermediate stages are designated by halves. In regard to the relative lime contents of plants these designations are used according to the following basis:

1 designates lime content of 0–0.74 per cent, inclusive
1½ designates lime content of 0.75–1.11 per cent, inclusive
2 designates lime content of 1.12–1.49 per cent, inclusive
2½ designates lime content of 1.50–1.86 per cent, inclusive
3 designates lime content of 1.87–2.24 per cent, inclusive
3½ designates lime content of 2.25–2.61 per cent, inclusive
4 designates lime content of 2.62–2.99 per cent, inclusive
4½ designates lime content of 3.00–3.36 per cent, inclusive
5 designates lime content of 3.37 per cent and over.

It is now to be recalled that the lime content of a plant and its rate of growth are two factors, both of which operate in the same direction as regards the lime requirement of a plant. The higher the lime content and the higher the rate of growth the greater will be the lime requirement of the plant. The feeding power of a plant is, however, a factor which works in the opposite

direction to the other two just mentioned. The greater the feeding power of a plant, the other two factors remaining constant, the lower will be the lime requirement of a plant. The lime requirement of a plant is thus determined by three factors, two of which operate in one direction and a third which operates in the opposite direction. The resultant of the three factors indicates the lime requirement.

Before describing the method of obtaining and using this resultant to determine the lime requirement, it is important to note as a fundamental basis, that a high feeding power will entirely offset both a high lime content and a high rate of growth, resulting in a low or medium lime requirement. The cowpea and buckwheat are excellent examples of this. If the lime content, rate of growth, and feeding power are all medium, then, of course, the lime requirement will also be medium and is represented by the figure 3. Using this simple case as a basis for calculation, the designations for lime requirements of other plants may be determined as follows:

If in this same case the factor "feeding power" were lower than 3, the lime requirement would rise above 3 accordingly, and if higher than 3, it would drop below 3 accordingly. The amount of rise or drop is determined by a comparison of the figure which represents the feeding power with a collective figure which represents both the lime content and the rate of growth. This collective figure represents what may be called *the required rate of lime delivery* and being dependent on both lime content and rate of growth is obtained as a relative figure by taking the average of the two. The product of the two is not taken since only relative figures are dealt with.

The method of calculation is best explained and illustrated by giving a few specific examples as follows:

In the case of sweet clover the relative lime content is represented by 3 and the relative rate of growth is estimated at 5, and hence the required rate of lime delivery is represented by 4, which is the average of the two. The feeding power is estimated at $3\frac{1}{2}$, which is lower by $\frac{1}{2}$ than the required rate of lime delivery and hence $\frac{1}{2}$ must be added to the adopted basial figure 3, giving $3\frac{1}{2}$ (medium to high) which represents the lime requirement of sweet clover as obtained by this method of calculation. Similarly, in the case of alsike clover the required rate of lime delivery is found to be 3 and the feeding power is estimated at $3\frac{1}{2}$, which is larger by $\frac{1}{2}$ than the required rate of lime delivery, and hence $\frac{1}{2}$ must be subtracted from the basial figure 3, giving $2\frac{1}{2}$ (low to medium), which represents the lime requirement of alsike clover as obtained by this method of calculation. In a few cases the figures obtained are a little greater than 5 or a little less than 1, but since 5 and 1 are taken to represent the extremes, they are used in these cases in place of the greater or smaller figures. When quarter and three-quarter numbers have resulted from calculations, they have been changed to the half and full numbers, respectively, since such small differences are meaningless when based to a considerable extent on estimates.

If the figures which were obtained in this way, and which are given in the last two columns of table 1, are examined, it is found that there is a remarkably close relationship between the lime requirements of plants and their response to the liming of acid soils, or reciprocally to their ability to grow on acid soils.

A brief discussion of the data relating to the different groups of plants follows:

In the case of the legumes all degrees of lime requirements are found the same as in the case of the non-legumes. The clovers present an interesting group of plants in this respect and the differences in lime content, rate of growth and feeding power for lime adequately explain their differences in lime requirement and ability to grow on acid soils. The same may be said to be true with the other legumes. As is evident, it is entirely unnecessary to postulate that the main effects of soil acidity on legumes are directly traceable to effects on the symbiotic nitrogen-fixing bacteria, or to a destructive or toxic action on the roots.

The cereals present another very interesting group of plants and the higher lime requirement of barley than of the others is also explained.

The hay and pasture grasses are a group of plants whose lime requirements are more similar than of any other group and in many cases it is difficult to estimate their relative feeding power except by resorting to their ability to grow on poor soils, which, however, is quite a reliable index.

Of the fiber crops, cotton and flax are known to have a low lime requirement while that of hemp is about medium. The reasons for this are found in the figures of table 1.

In the miscellaneous crops, various degrees of lime requirements are represented and quite satisfactorily explained.

Most of the garden crops have rather high lime requirements and undoubtedly the more general use of lime for many of these crops would materially cut down the amount of manure required in gardening and truck farming. In the case of a few plants like the carrot and radish, there is a discrepancy of a whole point in the two sets of figures but this may easily be due to lack of data for basing estimates or other special effects of soil acidity. The reason why the cranberry and strawberry grow so well on acid soils is clearly indicated.

There is very little data available regarding the lime content of weeds and only the case of sorrel is given. The reason for its low lime requirement is clearly indicated, although it does have nearly a medium lime content.

A few varieties of trees also are included although data for making estimates are quite meagre. The resulting figures are, however, quite significant, especially in connection with the relation of native tree vegetation to native soil fertility. The trees with the higher lime requirements are found growing on the better soils.

These findings thus substantiate the theory which has been proposed; namely, the main specific injury of soil acidity is that it prevents plants, especially those with high lime requirements and relatively weak feeding powers, from getting the lime from the soil at a sufficiently rapid rate to meet their needs.

Exact concordance between the two columns of figures is of course not to

TABLE 1

The Relation of the Lime Requirements of Plants to their Degree of Response to the Liming of Acid Soils, or Reciprocally to their Ability to grow on Acid Soils

KIND OF PLANT	CALCIUM OXIDE OR LIME IN MATERIAL ANAYZED					RELATIVE RATE OF PLANT GROWTH	REQUIRED RATE OF LIME DELIVERY	FEEDING POWER OF PLANT FOR LIME	LIME REQUIREMENT OF PLANT	RESPONSE OF PLANT TO LIMING, OR RECIPROCALLY ABILITY TO GROW ON ACID SOILS
	Character of material analyzed	Number of analyses	Reported by	Per cent (dry basis)	Relative amount					
I. LEGUMES										
Alfalfa, *Medicago sativa*	Plant in bloom	12	(49)	3.00	4½	4½	4½	2	5	5
Clover, sweet, *Melilotus alba*	Hay	1	(2)	1.94	3	5	4	3½	3½	4
Clover red, *Trifolium pratense*	Plant in bloom	113	(49)	2.40	3½	4	4	3½	3½	3½
Clover, mammoth, *Trifolium medium*	Plant in bloom	3	(49)	2.09	3	4	3½	3½	3	3
Clover, alsike, *Trifolium hybridum*	Plant in bloom	3	(49)	1.62	2½	3½	3	3½	2½	2½
Clover, crimson, *Trifolium incarnatum*	Plant in bloom	4	(49)	1.92	3	3½	3½	4	2½	2
Clover, white, *Trifolium repens*	Plant in bloom	4	(49)	2.21	3	3	3	4	2	2
Clover, Japanese, *Lespedeza striata*	Plant in bloom	1	(49)	1.28	2	3	2½	3	2½	2
Beans, *Phaseolus vulgaris*	Vines	4	(49)	1.32	2	3½	3	4	2	2
Cowpea, *Vigna unguiculata*	Hay	1	(2)	2.70	4	4	4	5	2	2
Lupine, blue, *Lupinus hirsitus*	Hay	3	(49)	1.05	1½	4	3	5	1	1½
Peas, garden, *Pisum sativum*	Vines	6	(49)	3.12	4½	3½	4	2½	4½	4
Peanuts, *Arachis hypogaea*	Vines	1	(6)	2.08	3	3	3	3	3	3
Soybean, *Glycine hispida*	Hay	2	(2)	2.76	4	4	4	4	3	3
Serradella *Ornithopus sativus*	Plant in bloom	3	(49)	2.19	3	2	2½	3	2½	2
Vetch, common, *Vicia sativa*	Green plant	25	(49)	1.96	3	3	3	4	2	2½
II. NON-LEGUMES										
a. Cereals										
Barley, *Hordeum sativum*	Before bloom	3	(49)	0.95	1½	4	3	3	3	3½
Buckwheat, *Fagopyrum esculentum*	In bloom	7	(49)	3.33	4½	4½	4½	5	2½	2

Corn, *Zea mays*	In bloom	7	(49)	0.83	1½	5	3½	3½	3	2½
Oats, *Avena sativa*	Before bloom	9	(49)	0.53	1	4	2½	4	1½	1½
Rice, *Oryza sativa*	Entire plant	1	(28)	0.23	1	2	1½	2½	2	2
Rye, *Secale cereale*	Before bloom	1	(49)	0.51	1	4	2½	4	1½	1
Wheat (winter), *Triticum vulgare*	Before bloom	2	(49)	0.48	1	3	2	2½	2½	2
b. Hay and pasture grasses										
Italian rye-grass, *Lolium Italicum*	Hay stage	2	(2)	1.07	1½	3½	2½	4	1½	2
Johnson-grass, *Sorghum halepense*	Hay stage	1	(49)	0.62	1	4	2	4	1	2
Kentucky blue-grass, *Poa pratensis*	Hay stage	4	(49)	0.42	1	3	2	2½	2½	2½
Meadow Fescue, *Festuca pratensis*	Hay stage	4	(2)	0.57	1	3	2	3½	1½	2
Millet (species not stated)	Hay stage	1	(11)	0.46	1	3½	2½	3½	2	2
Orchard-grass, *Dactylis glomerata*	Hay stage	4	(2)	0.46	1	3½	2½	3½	2	2½
Perennial rye-grass, *Lolium perenne*	Hay stage	2	(2)	0.64	1	3	2	3½	1½	2
Redtop, *Agrostis vulgaris*	Hay stage	4	(2)	0.57	1	3	2	3½	1½	1
Timothy, *Phleum pratense*	Hay stage	2	(2)	0.71	1	3½	2½	3½	2	2½
c. Fiber crops										
Cotton, *Gassypium hirsutum*	Entire plant	1	(49)	0.75	1½	3	2½	3½	2	1½
Flax, *Linum usitatissimum*	Entire plant	1	(49)	0.75	1½	1½	1½	1½	1½	1
Hemp, *Cannabis sativa*	Entire plant	1	(49)	0.76	1½	5	3½	3½	3	3
d. Miscellaneous crops										
Hops, *Humulus lupulus*	Entire plant	3	(49)	2.30	3½	3	3½	3	3½	3½
Potato, common *Solanum tuberosum*	Vines	6	(49)	2.80	4	2	3	3	3	2½
	Tubers	59	(49)	0.10						
Potato, sweet, *Ipomoea batatas*	Vines	1	(3)	1.70	2½	2½	2½	3	2½	2
Rape, *Brassica napus*	In bloom	6	(49)	1.78	2½	3½	3	3	3	3½
Sugar beet, *Beta vulgaris*	Leaves	25	(49)	3.00	4½	2½	3½	2	4½	5
	Roots	149	(49)	0.23						
Sorghum, *Sorghum vulgare, saccharatum*	Entire plant	1	(36)	0.26	1	4	2½	4	1½	2
Sugar cane *Saccharum officinarum*	Entire plant	13	(49)	0.19	1	4	2½	3½	2	2
Tobacco, *Nicotiana tabacum*	Entire plant	1	(42)	3.40	5	3	4	2	5	4
Turnip, *Brassica rapa*	Leaves	10	(49)	3.83	5	2	3½	2½	4	3
	Roots	32	(49)	0.85						

TABLE 1—*Continued*

KIND OF PLANT	CALCIUM OXIDE OR LIME IN MATERIAL ANALYZED					RELATIVE RATE OF PLANT GROWTH	REQUIRED RATE OF LIME DELIVERY	FEEDING POWER OF PLANT FOR LIME	LIME REQUIREMENT OF PLANT	RESPONSE OF PLANT TO LIMING OR RECIPROCALLY ABILITY TO GROW ON ACID SOILS
	Character of material analyzed	Number of analyses	Reported by	Per cent (dry basis)	Relative amount					
e. Garden crops, etc.										
Cabbage, *Brassica oleracea, capitata*	Leaves	2	(49)	5.93	5	2½	4	2½	4½	4
	Heart	3	(49)	1.17						
Carrot, *Daucus carota*	Leaves	8	(49)	4.45	5	2½	3½	2	4½	3½
	Root	11	(49)	0.62						
Cauliflower, *Brassica oleracea, botrytis*	Leaves and stalk	1	(49)	2.04	3	2	2½	1	4½	4
Cranberry, *Vaccinium macrocarpon*	Leaves		(2)	0.40	1	1	1	3	1	1
	Fruit		(2)	0.03						
Celery, *Apium graveoleus*	Young shoots	1	(49)	1.88	2½	3	3	1	5	5
Grape, *Vitis*	Leaves	18	(49)	2.55	3½	3½	3½	3	3½	3
Kohl-rabi, *Brassica oleracea, caulo-rapa*	Leaves	1	(49)	4.64	5	2	3½	2½	4	4
Lettuce, *Lactuca sativa*	Head lettuce	3	(49)	2.65	4	3½	4	2½	4½	5
Onion, *Allium cepa*	Leaves	1	(49)	3.63	5	1	3	1½	4½	5
	Bulb	2	(49)	1.21						
Rhubarb, *Rheum rhaponticum*	Leaves	1	(49)	0.32	1	4	2½	3	2½	2½
	Stalks	1	(49)	1.45						
Radish, *Raphanus sativus*	Leaves	1	(49)	4.30	5	3	4	3	4	3
	Root	1	(49)	1.38						
Spinach, *Spinacia oleracea*	Top	2	(49)	1.96	3	3	3	2	4	5
Strawberry, *Fragaria chiloensis*	Vines		(2)	1.22	2	1½	2	3½	1½	2
	Fruit		(2)	0.07						
f. Weeds										
Sheep sorrel, *Rumex acetosella*	Whole plant	1	(49)	1.63	2½	2	2½	4	1½	1
g. Trees										
Chestnut	Leaves	1	(49)	2.25	3½	2½	3	3	3	3
Mulberry	Leaves	1	(49)	5.14	5	2	3½	2	4½	4½
Oak	Leaves	1	(49)	0.91	1½	2½	2	3½	1½	2
Pine	Needles	8	(49)	0.52	1	2	1½	3½	1	1

be expected in all cases, partly because of the meagre data available in some cases for basing conclusions, and partly because of the intervention of other factors of general soil fertility which in some cases may be much greater than others. The writer thus wishes to state clearly that many minor errors surely exist in the figures of table 1, which only careful investigation can eliminate. On the basis of the data available the validity of the theory in the main, however, seems certain.

The foregoing considerations and conclusions are especially important in formulating a simple, practical, and rational system of determining the amount of lime which should be applied under practical farming conditions. It is thus evident that the amount of lime which should be applied, assuming that a good quality is to be used, depends upon the following factors:

1. Lime requirement of crop or crops to be grown.
2. Degree of acidity of the soil.
3. Fertility of the soil.

How these factors may be combined into a single usuable quantity will be discussed in the succeeding articles.

SUMMARY

With a few exceptions agricultural plants grow best on soils well supplied with readily available lime. To be readily available, lime may exist either as the carbonate, as an easily hydrolyzable silicate or salt, or as a constituent of easily decomposable organic matter. The classification of agricultural plants as being lime-loving, lime-avoiding and indifferent, or as being acid-intolerant, acid-tolerant and indifferent, leads to confusion and gives the wrong impression regarding the relation of lime and soil acidity to plant growth. The subject is very complex and, as has been indicated, soil acidity has many indirect and general influences on soil fertility due to its effect on physical, chemical, and biological conditions of the soil.

It is well known that an acid condition is unfavorable to the highest development of desirable physical and biological soil conditions. An acid condition usually lowers the availability of nearly all the essential elements. On the other hand, soil acidity usually favors the accumulation and solubility of toxic organic and inorganic substances. Among these toxic substances soluble aluminum salts have been noted by a number of investigators, and possibly in some cases manganese salts should also be considered. In certain unusual cases of soil acidity sufficient amounts of these toxic substances may be present to be very harmful to some plants. The relation of soil acidity and liming to malnutrition due to a lack of iron in the plant, to plant diseases and to plant competition also need to be considered in a few special cases, particularly in cases where soil acidity appears to be a favorable condition.

Besides the indirect influences which affect all plants, and some probably to a considerable extent, soil acidity has a specific influence which affects

some plants like alfalfa and sugar beets very much more than others, like cowpeas, potatoes, and oats. At various times this specific influence of soil acidity has been ascribed to at least three causes: viz., (a) its effect on the supply of available calcium needed by plants as direct plant-food material, (b) its effect on the symbiotic nitrogen-fixing bacteria, (c) and its toxic or destructive effect on the root tissues of plants.

The supply of available calcium in all forms becomes less as soils become acid, but usually there is still sufficient present to furnish that needed as direct plant-food material. Since the symbiotic nitrogen-fixing bacteria live in the nodules, soil acidity cannot affect them directly, except before they enter into symbiosis, when it may lessen their activity and delay the time of infection. Since the relation of non-legumes to soil acidity runs parallel with the relation of the legumes, it follows that the direct influence is not on the legume bacteria but on the plants themselves.

That this direct effect on the plants is not often due to a destructive action of the acidity on the root tissues is evident from the fact that experiments have shown that plant roots are unaffected by solutions of a higher acidity than that of most acid soil solutions. This is further substantiated by the fact that the acidity (H-ion concentration) of the sap of most plants is of the same order as that of the soil solution of most acid soils, indicating that similar processes are probably at work in the two cases, as a result of certain analogous conditions which exercise a regulatory function in this respect. It is undoubtedly largely proteins in the case of plants, and collodidal organic and inorganic matter, especially silicates, in the case of soils which act as "buffers" and thus bring about this regulation of acidity to a considerable extent, preventing rapid changes and unusually high degrees of soluble acidity.

In most cases it thus appears that the *main* specific harmful influence of soil acidity on certain plants is not due to any of the three suggested reasons, but to its influences in preventing these plants from getting at a sufficiently rapid rate the calcium as the carbonate or bi-carbonate which is needed to neutralize and precipitate certain acids in the plants themselves, which are probably largely by-products, produced as the result of certain vital reactions in the growth of plants. If calcium in these forms is not furnished at a sufficiently rapid rate, then the rate of these reactions is lowered accordingly as is also the rate of plant growth.

Each species of plant has a certain lime requirement which must be satisfied for maximum plant growth and this lime requirement is defined by the writer as follows: *The expression "lime requirement" of a plant refers to the actual lime needs of theplant itself, especially as to the ease and rate at which lime must be secured from the soil by the plant for normal growth.* Thus if a plant has a high lime requirement, then the solution and delivery must be rapid and easy in order to meet the needs of the plant.

The three main factors which determine the lime requirement of a plant

are: (a) lime content, (b) rate of growth, and (c) feeding power of the plant for lime. The first two factors operate in one direction while the third operates in the opposite direction. That is, the higher the lime content and the rate of growth, the higher will be the lime requirement, and conversely. Also, the higher the feeding power for lime, the lower will be the lime requirement, and conversely. The resultant of these three gives the lime requirement of the plant. A simple method of expressing these factors and obtaining the resultant is described in this article.

A table is also given in which are expressed the lime requirements of 62 species of plants as obtained by this method. These lime requirements are compared with corresponding figures which represent the relative response of these plants to the liming of acid soils, or reciprocally to their ability to grow on acid soils. The comparison reveals a close correspondence and hence substantiates the theory which has been proposed that, usually, the main specific injury of soil acidity is that it prevents plants, especially those with high lime requirements and relatively weak feeding powers, from getting the lime from the soil at a sufficiently rapid rate to meet their needs. This is further substantiated by the parallel relation found between the amount of growth of alfalfa on acid soils and the amount of calcium which could be extracted with carbonated water from these soils. These considerations are especially important in formulating a practical system of using lime, especially as regards the amount to be used which, as is discussed in detail, is dependdent on the lime-requirement of the crop, the degree of acidity of the soil, and the fertility of the soil.

REFERENCES

(1) Abbott, J. B., Connor, S. D., and Smalley, H. R. 1913 Soil acidity, nitrification, and the toxicity of soluble salts of aluminum. Ind. Agr. Exp. Sta. Bul. 170, p. 329–374.

(2) Beal, W. H. 1889 Compilations of analyses of fodder articles, fruits, sugar, producing plants, dairy products, etc. *In* Mass. State Agr. Exp. Sta. 7th Ann. Rpt. (1889), p. 291–313.

(3) Blair, A. W. 1908 Total ash and lime in some feeding materials and fruits. *In* Fla. Agr. Exp. Sta. Ann. Rpt. 1908, p. 46.

(4) Briggs, L. J., and Breazeale, J. F. 1917 Availability of potash in certain orthoclase-bearing soils as affected by lime or gypsum. *In* Jour. Agr. Res., v. 8, no. 1, p. 21–28.

(5) Contejean 1881 Geographie botanique. Influence du terrain sur la vegetation. 143 p. Paris. (From Hilgard, E. W. (22), p. 521.)

(6) Corbett, L. C. 1907 Peanut. *In* Bailey's Cyclopedia of American Agriculture, v. 2, p. 519.

(7) Frear, W. 1915 Sour soils and liming. Pa. Dept. Agr. Bul. 261, p. 101.

(8) Fred, E. B., and Graul, E. J. 1916 The gain in nitrogen from growth of legumes on acid soils. Wis. Agr. Exp. Sta. Res. Bul. 39, p. 1–42.

(9) Fred, E. B., and Hart, E. B. 1915 The comparative effect of phosphates and sulphates on soil bacteria. Wis. Agr. Exp. Sta. Res. Bul. 35, p. 35–66.

(10) FRED, E. B., AND LOOMIS, N. E. 1917 Influence of hydrogen-ion concentration of medium on the reproduction of alfalfa bacteria. *In* Jour. Bact., v. 2, no. 6, p. 629–633.

(11) FORBES, E. B., BEEGLE, F. M., AND MENSCHING, J. E. 1913 Mineral and organic analyses of foods. Ohio Agr. Exp. Sta. Bul. 255, p. 211–232.

(12) GEDROITZ, K. K. 1910 The effect of acids, alkalis, and some inorganic salts on plants. *In* Zhur. Opuitn. Agron. (Russ. Jour. Exp. Landw.), v. 11, no. 4, p. 544–578, no. 5, p. 641–678.

(13) GILE, P. L. 1911 Relation of calcareous soils to pineapple Chlorosis. Porto Rico Agr. Exp. Sta. Bul. 11, 45 p.

(14) GILE, P. L., AND AGETON, C. N. 1914 The effect of strongly calcareous soils on the growth and ash composition of certain plants. Porto Rico Agr. Exp. Sta. Bul. 16, 45 p.

(15) GILLESPIE, L. J. 1916 The reaction of soil and measurements of hydrogen-ion concentrations. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, p. 8–16.

(16) HALL, A. D. 1908 The Soil, p. 40. E. P. Dutton Co., New York.

(17) HALL, A. D. 1908 Fertilizers and Manures, p. 253. E. P. Dutton Co., New York.

(18) HALL, A. D. 1910 The adaptation of the plant to the soil. *In* Jour. Roy. Hort. Soc. (London), v. 36, p. 1–21.

(19) HARTWELL, B. L., AND DAMON, S. C. 1914 The comparative effect on different kinds of plants of liming an acid soil. R. I. Agr. Exp. Sta., Bul. 160, p. 407–446.

(20) HARTWELL, B. L., AND PEMBER, F. R. 1918 Aluminum as a factor influencing the effect of acid soils on different crops. *In* Jour. Amer. Soc. Agron., v. 10, no. 1, p. 45.

(21) HARTWELL, B. L., AND PEMBER, F. R. 1907 The relation between the effects of liming and of nutrient solutions containing different amounts of acid, upon the growth of certain cereals. *In* R. I. Agr. Exp. Sta. 20th Ann. Rpt. (1907), p, 358–380.

(22) HILGARD, E. W. 1905 Soils, p. 365. Macmillan Co., New York.

(23) HOAGLAND, D. R. 1917 The effect of hydrogen and hydoxyl-ion concentration on the growth of barley seedlings. *In* Soil Sci., v. 3, no. 6, p. 547–560.

(24) JOHNSON, M. O. 1917 Manganese as a cause of the depression of the assimilation of iron by pineapple plants. *In* Jour. Indus. Engin. Chem., v. 9, no. 1, p. 47–49.

(25) LECLERC, J. A., AND BREAZEALE, J. F. 1908 Plant food removed from growing plants by rain or dew. *In* U. S. Dept. Agr. Yearbook 1908, p. 389.

(26) LYON, T. L., FIPPIN, E. O., AND BUCKMAN, H. O. 1916 Soils, Their Properties and Management, p. 537. Macmillan Co., New York.

(27) MACINTIRE, W. H., WILLIS, L. G., AND HARDY, J. I. 1914 The non-existence of magnesium carbonate in humid soils. Tenn. Agr. Exp. Sta. Bul. 107, p. 151–202, 2 figs.

(28) MCDONNELL, C. C. 1901 A chemical investigation of the rice plant and of the product and by-products of the rice industry. S. C. Agr. Exp. Sta. Bul. 59, 16 p.

(29) MORSE, F. W., AND CURRY, B. E. 1908 A study of the reactions between the manurial salts, clay, mucks, and soils. N. H. Agr. Exp. Sta. 19th and 20th Ann Rpts. (1908–09), p. 271–293.

(30) MORSE, F. W., AND CURRY, B. E. 1909 The availability of the soil potash in clay and clay loam soils. N. H. Agr. Exp. Sta. Bul. 142, p. 39–58.

(31) PETERSON, P. P. 1912 Effect of heat and oxidation on the phosphorus of the soil. Wis. Agr. Exp. Sta. Res. Bul. 19, p. 1–16.

(32) PFEIFFER, T., AND BLANCK, E. 1914 Die Kalkfeindlichkeit der Lupine. *In* Mitt. Landw. Inst. Breslau, Bd. 7, No. 2, p. 201–233.

(33) PIPAL, F. J. 1916 Red sorrel and its control. Ind. Agr. Exp. Sta. Bul. 197.

(34) RUPRECHT, R. W. 1915 Toxic effects of iron and aluminum salts on clover seedlings. Mass. Agr. Exp. Sta. Bul. 161, p. 125–129.
(35) SHARP, L. T., AND HOAGLAND, D. R. 1916 Acidity and adsorption in soils as measured by the hydrogen electrode. *In* Jour. Agr. Res., v. 7, no. 3, p. 123–145.
(36) SHOREY, E. C. 1906 The composition of some Hawaiian feeding stuffs. Hawaii Agr. Exp. Sta. Bul. 13, p. 16.
(37) SORENSEN, S. P. L. 1912 Ueber die Messung und Bedeutung der Wasserstoff ionen-koncentration bei biologischen Prozessen. *In* Ergeb. Physiol., v. 12, p. 393–532.
(38) STODDART, C. W. 1909 Soil acidity in its relation to lack of available phosphates. *In* Jour. Indus. Engin. Chem., v. 1, no. 2, p. 69–74.
(39) TRUESDELL, H. W. 1917 The effect of phosphorus on alfalfa and alfalfa bacteria. *In* Soil. Sci., v. 3, no. 1, 77–98 p.
(40) TRUOG, E. 1915 A new test for soil acidity. Wis. Agr. Exp. Sta. Bul. 249, p. 1–16.
(41) TRUOG, E. 1915 Soil acidity and methods for its detection. *In* Science, n.s., v. 42, no. 1084, p. 505–507.
(42) TRUOG, E. 1916 The utilization of phosphates by agricultural crops, including a new theory regarding the feeding power of plants. Wis. Agr. Exp. Sta. Res. Bul. 41, p. 34.
(43) TRUOG, E., AND SYKORA, J. 1917 Soil constituents which inhibit the action of plant toxins. *In* Soil Sci., v. 3, no. 4, p. 333–351.
(44) TRUOG, E. 1917 Letter on the avidity of soil acids. *In* Jour. Indus. Engin. Chem., v. 9, no. 11, p. 1072.
(45) WAY, J. T., AND OGSTON, G. 1870 Ash analyses of plants. *In* Wolff, E. (49), pt. 1, p. 178.
(46) WHEELER, H. J. 1913 Manures and Fertilizers, p. 307. Macmillan Co., New York.
(47) WHITE, J. W. 1916 Concerning the growth and composition of clover and sorrel as influenced by varied amounts of limestone. *In* Pa. Agr. Exp. Sta. Ann. Rpt. 1913–14, p. 46–67.
(48) WILCOX, E. V., AND KELLEY, W. P. 1912 The effect of manganese on pineapple plants. Hawaii Agr. Exp. Sta. Bul. 28, 20 p.
(49) WOLFF, E. 1870 and 1880 Aschen Analysen, pt. 1, 1870, 194 p., pt. 2, 1880, 170 p., Wiegant and Hempe, Berlin.

THE EFFECT OF HEAT ON SOME NITROGENOUS CONSTITUENTS OF SOIL

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Received for publication January 7, 1918

In the investigations of the effect of heat on soil the only nitrogenous constituents existing as such which have been determined quantitatively are ammonia and nitrates. Kelley and McGeorge (4) also determined the change in monoamino, diamino and amid nitrogen in the hydrochloric acid hydrolysed, untreated soil and the same soil after heating to 200°C. In this laboratory, methods have been developed for the determination of amino acid, nitrogen and soluble non-protein nitrogen (12, 13). These constituents had previously never been determined in soils by the use of such reagents as precluded their formation from more highly organized material or their partial destruction during the procedure for the determination. A method for the determination of ammonia (11) which gives more nearly absolute results than those usually used also has been worked out. It was because of these and other considerations that the work herein reported was undertaken.

For an extensive article on the literature on soil sterilization, the paper by Kopeloff and Coleman (6) appearing in a recent number of this journal should be consulted. Only the more important investigations having a direct bearing upon the phases of the subject taken up by us will be discussed here.

Richter in 1896 (14) found that heating a soil to 100°C. increased the amount of nitrogen soluble in water and dilute hydrochloric acid, while the water-soluble organic matter was increased about 300 per cent.

Nitrification was found by Deherain and DeMoussy (2) to be inhibited completely in soils heated at 120°C. for 1 hour in the autoclave. Upon inoculation of the heated soil with fresh soil an abnormally large amount of nitrates was produced. Pickering (9, 10) found that heating soils caused an increase in the total soluble organic matter. Upon inoculation of the heated soil and incubation for a few weeks, this excess soluble organic matter was decreased somewhat. He also concluded that the action of the common volatile antiseptics caused about the same chemical change in a soil as heat at 60–75°C.

Russell and his associates (15, 16) have carried out more comprehensive work along these lines than any other investigators. The results of their researches have led them to propose the theory that protozoa are an important limiting factor of bacterial growth. The conclusions to be drawn from their work having direct bearing on the subject matter of this paper, are as

follows. Partial sterilization of the soils, that is, heating at 98°C. or treatment with toluene, caused an increase in the amount of ammonia. This amount of ammonia was further increased to a relatively great extent by incubation of the partially sterilized soil. Nitrates were not changed by heat at 98°C. nor was there any appreciable change after standing in the amount present either in the uninoculated soil or the soil inoculated with a fresh soil. Their contention was that nitrifying organisms would not work in the heated soil. "Unstable nitrogen compounds" were increased by heat at 98°C. to about the same relative extent as ammonia. They were also increased by subsequent incubation of the soil but to a less extent than the ammonia. The "unstable nitrogen compounds" were determined by distillation of the soil with magnesia at 100°C. for an arbitrarily fixed length of time. The amount of ammonia thus formed minus that formed by distillation of the soil at 38°C. was taken to be unstable nitrogen. The amount of humus present in the heated soil was somewhat less than in the unheated.

Lyon and Bizzell (7, 8) studied the changes in soluble matter of soils brought about by steaming at 2 atmospheres and the further changes at the end of various lengths of time after inoculation with fresh and with steamed soils. As they point out, the results obtained on some soils are quite inconsistent when compared with others, but the general conclusions to be drawn from their work are as follows. Steaming invariably increased the amount of soluble organic matter and ammonia and decreased the nitrates. After inoculation with fresh soil and incubation for periods ranging from 5 to 19 weeks, nitrates were often decreased and never increased but to a slight extent. Ammoniacal nitrogen was invariably increased by incubation for periods up to 19 weeks, while total soluble matter was decreased. One sample of steamed soil was inoculated with fresh soil and allowed to incubate for 6½ months. Nitrates were greatly increased, total soluble matter was increased and ammonia was considerably decreased. These results were just opposite to those found in the same soil after incubation for the shorter periods.

Schreiner and associates (17) found that heating at 135°C. in the autoclave for 3 hours increased the amount of xanthine and hypoxanthine when they were found in the untreated soil. When they were not found in the unheated soil appreciable amounts could be separated from the heated soil. Cytosin, guanine and arginine not found in the unheated soil were isolated after heating the soil. Pentosans, histidine, creatinine and dihydroxystearic acid were found to be increased by the treatment in the autoclave. The amount of nucleic acid was lessened.

EXPERIMENTAL

The plan of our work was as follows:

Large samples of four soils were put through a ⅛-inch mesh sieve and each sample thoroughly mixed. Several small samples of each of the soils of 350 gm. each were weighed into 500-

cc. Florence flasks, cotton plugs inserted and then subjected to the following treatments: Two hours at 100°C., dry heat; 2 hours at 200°C., dry heat; the same treatment and then inoculated with fresh soil emulsion and incubated for 10 weeks, the same treatment and then inoculated as above and incubation for 20 weeks; 15 pounds in the autoclave for 3 hours; 10 pounds in the autoclave for 3 hours per day for 3 successive days; the same treatment, inoculated as above and incubated for 10 weeks; the same treatment, inoculated as above and incubated for 20 weeks. All treatments and incubations were carried out in duplicate. Before incubation the soils were made up to the optimum moisture content and maintained at that throughout the incubation period. After the soils had been subjected to the various treatments they, together with duplicate samples of the untreated soils, and the inoculated soils, after they had incubated the specified length of time, were subjected to analysis as described below.

METHODS OF ANALYSIS

Loss on ignition. Approximately 2 gm. of soil were accurately weighed into covered porcelain crucibles. The crucible and contents were then placed in the oven at 100–105°C. for 3 hours and then weighed again. This gave the amount of hygroscopic moisture. These results are not recorded here because of the fact that the soils were not held under uniform conditions between the time of the completion of the respective treatments and the weighing into the crucibles. The determination, however, was necessary, in order to calculate the results to the oven-dry basis. After obtaining the oven-dry weight the crucibles were placed in the muffle furnace and heated for 2 hours or more at a bright red heat. All determinations were made in duplicate.

Nitrate nitrogen. Nitrates were determined by the modification of the aluminum reduction method as proposed by Burgess (1) using the apparatus proposed by us (11).

Ammonia nitrogen. This was determined by the method developed in this laboratory (11).

Amino acid nitrogen. The amino acid nitrogen was determined according to our modification (12) of the Kober (5) method.

Soluble non-protein nitrogen. This group of compounds were determined by the Greenwald (3) method as modified by us for use on soil extracts (13).

DESCRIPTION OF THE SOILS

Calhoun silt loam. This soil was obtained from Van Buren County, Iowa, which is situated in the Southern Iowa loess soil area. It is a fairly fertile soil, acid in reaction and rather low in organic matter.

Miami silt loam I. This was obtained from one of the station orchards which is situated in the Wisconsin drift soil area. This soil is similar to the Calhoun silt loam but contains a little sand, is lighter in color and contains less organic matter.

Miami silt loam II. This comes from a different place in the same orchard

as the above soil. It is darker in color and contains more organic matter than the other sample of Miami silt loam.

Peat soil. This soil was secured from a peat spot in one of the experiment station pastures. It is a typical peat soil, black in color and quite light in weight. The amount of organic matter present is shown below by its loss on ignition.

In table 1 are found the results obtained with the Van Buren County soil. The values for the loss on ignition have been calculated on the basis of the ignited soil. The reason for adopting this standard instead of the oven-dry basis was that some loss of organic material was caused by the more severe

TABLE 1

Calhoun silt loam soil

TREATMENT	LOSS ON IGNITION	TOTAL NITROGEN	PER CENT OF TOTAL NITROGEN							
			Ammonia		Amino acid		Nitrate		Soluble non-protein nitrogen	
			Original basis	Final basis	Original basis	Final basis	Original basis	Final basis	Original basis	Final basis
	per cent	*per cent*								
Air-dry	5.80	0.181	0.60		0.270		0.325		14.45	
100°C.; 2 hours	5.47		0.89		0.230		0.450		16.70	
10 pounds; 9 hours	5.88	0.177	3.01	3.08	0.530	0.54	0.895	0.915	22.80	23.6
15 pounds; 3 hours	5.59	0.179	1.34	1.35	0.430	0.44	0.660	0.670	17.10	17.3
200°C.; 2 hours	4.67	0.175	12.70	13.10	0.890	0.92	0.120	0.120	30.30	31.3
10 pounds; 9 hours; 10 weeks incubation			6.14	6.28	0.415	0.42	2.090	2.130	20.80	21.3
10 pounds; 9 hours; 20 weeks incubation			4.89	5.00	1.150	1.18	2.650	2.700	20.80	21.3
200°C.; 2 hours; 10 weeks incubation			17.05	17.60	0.340	0.35	1.280	1.320	30.60	31.6
200°C.; 2 hours; 20 weeks incubation			17.20	17.80	1.730	1.69	2.320	2.400	32.50	33.6

treatments, and hence it would be incorrect to express the percentages on the oven-dry weight of each soil. It is seen that the percentages of the various nitrogenous constituents are expressed on two bases. The results in the "original basis" columns are expressed in per cent of the total nitrogen originally in the soil. The "final basis" results represent the per cent of the total nitrogen left in the soil. No nitrogen was lost by the 100°-heated soil and it was assumed that no change in the total nitrogen was occasioned by the incubation. This probably is not strictly correct but the change in total nitrogen in the short incubation periods of 10 and 20 weeks would be very slight.

The figures giving the loss on ignition may be taken to represent the amount

of organic matter in the soils. There is seen to be some change in the organic matter of the soil by all the treatments but perhaps the only one great enough to be of any significance is the 200° treatment, and here the change from 5.80 to 4.67 per cent represents a loss of 19.5 per cent of the organic matter.

The next column of the table gives the per cent of nitrogen in the soil from the various treatments. Here none of the treatments show much of a loss. The results for the soil heated at 15 pounds might well be considered to vary from the untreated soil by no more than the experimental error, but the other two figures are probably well without the error. The 200° treatment causes a loss of 3.3 per cent of nitrogen, considerably less than the total loss of organic matter. At 10 pounds, a smaller loss of 2.21 per cent was found.

The results for the changes in the nitrogenous constituents in the incubated soils will be taken up now. The results for the various heat treatments have been plotted and appear in figures 1 to 4, and their discussion will be taken up later.

The 3.01 per cent of ammoniacal nitrogen in the soil heated at 10 pounds more than doubled in 10 weeks. The amount then dropped somewhat at the end of the 20 weeks. Considering the nitrate results it is seen that not all of this drop is necessarily due to nitrification. In this connection it is interesting to observe that the increase in nitrates in the first 10 weeks was proportionate to the increase in ammonia. In the next 10 weeks, the nitrates increased somewhat. The amino acid nitrogen diminished somewhat in the first 10 weeks but increased to a considerable extent in the next period. From these results and the resuts for ammonia it would seem that ammonification in the first period proceeded quite rapidly at the expense of the amino acid, while in the second period ammonification decreased considerably, allowing a relatively large accumulation of amino acids. The soluble non-protein nitrogen diminished somewhat during the first 10 weeks and then remained stationary during the next period. This group of compounds, although its nature is largely unknown, represent compounds of relatively simple constitution. In the original communication (13) on the determinations of the soluble non-protein nitrogen, it is shown that such complex substances as egg albumin, edestin and casein do not appear to any extent whatever in this group when the soil to which the substances are added is immediately subjected to the analysis. It would seem that the bacteria would attack the compounds in this group rather than the more insoluble portion, but this contention is not borne out entirely by the figures for the analyses. The decrease in the per cent present caused by the incubation is less than the increase of ammonia and nitrate nitrogen. Then, too, the increase in ammonia and nitrates does not represent the total increase, for no doubt relatively large amounts of these compounds were assimilated by the bacteria. The changes taking place in the soils heated to 200° follow in a general way the similar changes in the soil heated in the autoclave at 10 pounds pressure. It is particularly interesting to note the small accumulation of nitrates in

spite of the large amount of ammonia present. Whether this is to be ascribed to actual absence of nitrification or whether the nitrates were being utilized by the bacteria as produced, it is difficult to state. The change in non-protein nitrogen caused by the incubation is only slight.

In table 2 the results for Miami Silt Loam Soil II are found.

From the figures for the loss on ignition it is seen that the soil heated to 200° lost 12.1 per cent of its organic matter. The changes caused by the other treatments are inappreciable. A temperature of 200° also caused a loss of 8.7 per cent of the nitrogen. Fifteen pounds pressure caused a loss of 8.1 per cent of nitrogen while 10 pounds caused a loss of 4.3 per cent of

TABLE 2

Miami silt loam soil II

TREATMENT	LOSS ON IGNITION	TOTAL NITROGEN	PER CENT OF TOTAL NITROGEN							
			Ammonia		Amino acid		Nitrate		Soluble non-protein nitrogen	
			Original basis	Final basis	Original basis	Final basis	Original basis	Final basis	Original basis	Final basis
	per cent	*per cent*								
Air-dry	4.78	0.161	0.89		0.48		0.68		17.1	
100°C.; 2 hours	4.84		1.26		0.57		0.60		21.6	
10 pounds; 9 hours	4.93	0.154	3.16	3.31	2.00	2.13	1.33	1.39	18.3	19.2
15 pounds; 3 hours	4.67	0.148	2.05	2.23	0.92	1.06	0.91	0.99	20.3	22.1
200°C.; 2 hours	4.20	0.147	9.99	10.90	1.22	1.34	0.28	0.31	24.7	27.1
10 pounds; 9 hours; 10 weeks incubation			5.70	5.96	0.45	0.47	1.82	1.90	22.2	23.2
10 pounds; 9 hours; 20 weeks incubation			4.27	4.47	1.25	1.31	2.70	2.82	22.6	23.6
200°C.; 2 hours; 10 weeks incubation			17.10	18.70	0.68	0.74	1.20	1.31	35.8	39.2
200°C.; 2 hours; 20 weeks incubation			20.40	22.50	0.95	1.04	0.97	1.06	34.8	38.1

nitrogen. It is difficult to say from what compounds this loss comes. Nitric acid as such would volatilize wholly at 200° and considerably at the 10-pound and 15-pound treatments. So far as we are able to find, the metallic nitrates are not decomposed at 200°. The loss of nitrates at 200°, therefore, is either to be accounted for by the assumption that at that temperature practically all of the metallic nitrates react with acids to give nitric acid which is volatilized or that the organic matter reduces the nitrates, probably to ammonia. Either assumption seems to be justified from the analytical data and from theoretical considerations. However, this would account for only a small part of the loss of total nitrogen. The chief loss of nitrogen probably comes from the volatilization of ammonia compounds, which in turn are formed by

the degradation of proteins, proteoses, peptones, etc. The purine bases and derivatives no doubt also contribute to the formation of ammonia. The change in the amounts of the different nitrogen compounds caused by the various heat treatments will be taken up later.

The change in ammonia, nitrate, and amino acid nitrogen in the soil treated at 10 pounds pressure caused by the incubation follows, in a general way, the same course as the respective changes in the soil previously discussed. It is interesting to note that the soil incubated 20 weeks contained more amino nitrogen than ammonia. So far as we can recall this is the only soil upon which analyses have been made which shows such a reversal of the usual condition.

TABLE 3

Miami silt loam soil I

TREATMENT	LOSS ON IGNITION	TOTAL NITROGEN	PER CENT OF TOTAL NITROGEN							
			Ammonia		Amino acid		Nitrate		Soluble non-protein nitrogen	
			Original basis	Final basis	Original basis	Final basis	Original basis	Final basis	Original basis	Final basis
	per cent	*per cent*								
Air-dry	3.75	0.130	1.04		0.36		1.43		17.95	
100°C.; 2 hours	3.54		1.16		0.52		1.41		17.7	
10 pounds; 9 hours	3.65	0.127	2.05	2.11	0.72	0.74	2.52	2.59	21.2	21.8
15 pounds; 3 hours	3.80	0.126	1.95	2.01	0.61	0.63	2.22	2.28	19.5	20.2
200°C; 2 hours	2.90	0.117	4.61	5.14	0.53	0.60	0.41	0.46	32.0	36.4
10 pounds; 9 hours; 10 weeks incubation			3.62	3.70	0.51	0.52	6.4	6.6	20.5	21.0
10 pounds; 9 hours; 20 weeks incubation			1.74	1.78	1.92	1.96	6.2	6.3	22.7	23.3
200°C.; 2 hours; 10 weeks incubation			12.0	13.30	0.63	0.70	2.02	2.25	38.15	42.4
200°C.; 2 hours; 20 weeks incubation			9.6	1.06	0.94	0.93	4.78	5.31	27.9	31.0

The changes in the incubated 200°-heated soils follow in some respects the same course as the respective changes in the Calhoun silt loam. The most noteworthy difference is to be found in the non-protein nitrogen which nearly doubles in the first 10 weeks but drops somewhat in the next period. Part of the first increase is due, of course, to the increased ammonia which would be in this fraction.

In table 3 are given the results obtained with the second sample of Miami silt loam soil. With this soil the loss of organic matter of the 200°-heated soil as shown by the loss on ignition was 22.6 per cent, as compared with a loss of 12.1 per cent for the other sample of this soil type. The difference is all the more noteworthy when it is recalled that the one showing the less loss

of organic matter contains at the start about 1 per cent more total organic matter. The loss of nitrogen of the soil the results of which are given in table 3 is 10.0 per cent.

With the exception of the nitrate nitrogen the changes which the various forms of nitrogen exhibit in the soil heated at 10 pounds and inoculated and incubated are in general similar to the changes undergone by the other sample of Miami soil. The nitrates show a gradual rise at the end of the first and second 10 weeks when compared with the amounts present before incubation. In the case of the 200°-heated soils the changes are not consistent when compared with the similar changes of the soils previously discussed. The amounts

TABLE 4

Peat soil

TREATMENT	LOSS ON IGNITION	TOTAL NITROGEN	PER CENT OF TOTAL NITROGEN							
			Ammonia		Amino acid		Nitrate		Soluble non-protein nitrogen	
			Original basis	Final basis	Original basis	Final basis	Original basis	Final basis	Original basis	Final basis
	per cent	*per cent*								
Air-dry	47.3	3.39	0.23		0.260		0.37		4.96	
100°C.; 2 hours	48.3		0.25		0.190		0.28		7. 7	
10 pounds; 9 hours	47.2	3.09	1.45	1.59	0.220	0.24	0.43	0.47	6.43	7.05
15 pounds; 3 hours	47.0	2.39	5.95	8.43	0.190	0.27	0.31	0.44	6.53	9.25
200°C.; 2 hours	41.6	2.42	1.35	1.90	0.170	0.24	0.05	0.07	10.90	15.30
10 pounds; 9 hours; 10 weeks incubation			1.28	1.40	0.135	1.48	3.92	4.30	6.66	7.31
10 pounds 9 hours; 20 weeks incubation			1.45	1.59	0.140	0.15	4.17	4.58	4.33	4.75
200°C.; 2 hours; 10 weeks incubation			1.02	1.42	0.110	0.15	3.76	5.26	5.78	8.10
200°C.; 2 hours; 20 weeks incubation			1.55	2.17	0.190	0.27	2.00	2.80	5.43	7.60

of ammonia and non-protein nitrogen have been increased by the incubation, whereas the amino acid nitrogen was diminished. The nitrates were increased by both periods of incubation but the increase was less for the longer period than for the shorter.

The results for the peat soil appear in table 4. It is observed that the treatment at 200° caused a loss of 12.05 per cent of organic matter, whereas by the same treatment 28.6 per cent of nitrogen was lost. This is a far greater loss of nitrogen than the three mineral soils have undergone. On first thought it would seem that there should be less loss of nitrogen from a peat soil, the nitrogen of which is generally held to be in a relatively inert, unavailable form. The reason for the greater loss probably lies in the fact that in the

peat soil there is a less proportionate amount of the minerals which act as adsorbents or absorbents for ammonia. For, as stated above, it probably is largely as ammonia that the nitrogen is lost. It is seen that in the peat soil the amount of ammonia found in the 200°-heated soils is low when compared with the similar amounts in the mineral soils.

When the results for the incubated soils are examined it is seen that but little change was brought about by the incubation. The results for the peat soil are striking in that respect when compared with the mineral soils. Probably the explanation of the small amount of change brought about by incubation is that the heat treatment released soluble toxic material to a great extent in this soil, so high in organic matter. Another interesting point seen on an inspection of the results for the peat soil is that in many cases the sum of the nitrate, ammonia and amino acid nitrogen is nearly equal to the soluble non-protein nitrogen. Certainly all the nitrate and amino acid nitrogen would be in the soluble, non-protein nitrogen fraction and the larger portion, if not all of the ammonia, would be in this fraction also. Therefore, unlike the other soils, the soluble non-protein nitrogen includes but little material other than ammonia, nitrate and amino acid nitrogen.

The changes in the various forms of nitrogen which the different heat treatments brought about have been plotted and appear in figures 1 to 4. As is seen from an inspection of the figures, treatments are plotted on the ordinates and the per cent change on the abscissa. Different standards for the different constituents have been used for the latter. Because the treatment points are separated from each other by regular periods on the ordinate, it is not meant to convey that actually, for instance, there is the same difference between air-dry soil and soil heated for 2 hours at 100°C., as there is between soil given the latter treatment and heated in the autoclave for 9 hours at 10 pounds pressure.

The results for the ammonia in the different soils are shown in figure 1. Heating at 100° for 2 hours is seen to increase slightly the amount of ammonia in all the soils and 9 hours at 10 pounds in the autoclave increases the ammonia still more. Fifteen pounds for 3 hours does not give such an increase in the mineral soils as the 10-pound treatment. Evidently the length of time of heating has quite an appreciable effect. We do not have an explanation of the great increase in ammonia in the peat soil. At 200° all the soils show an increase in ammonia when compared with the treatment at 15 pounds, except the peaty soil which shows a decrease. This decrease, as pointed out above, is probably largely due to the fact that the ammonia had volatilized from the peat soil to a considerable extent. While ammonia has no doubt been evolved from the mineral soils to a considerable extent at 200° also, yet the large amount of minerals in the latter soils makes necessary a greater accumulation of ammonia before appreciable volatilization takes place.

The amino acid results are shown in figure 2. The amino acid nitrogen in the two Miami silt loam soils was increased by heat at 100°, whereas

this form of nitrogen was decreased in the peat and Calhoun soils by the same treatment. Treatment at 10 pounds increased the amount of amino acid nitrogen in all the soils and at 15 pounds the amounts were decreased.

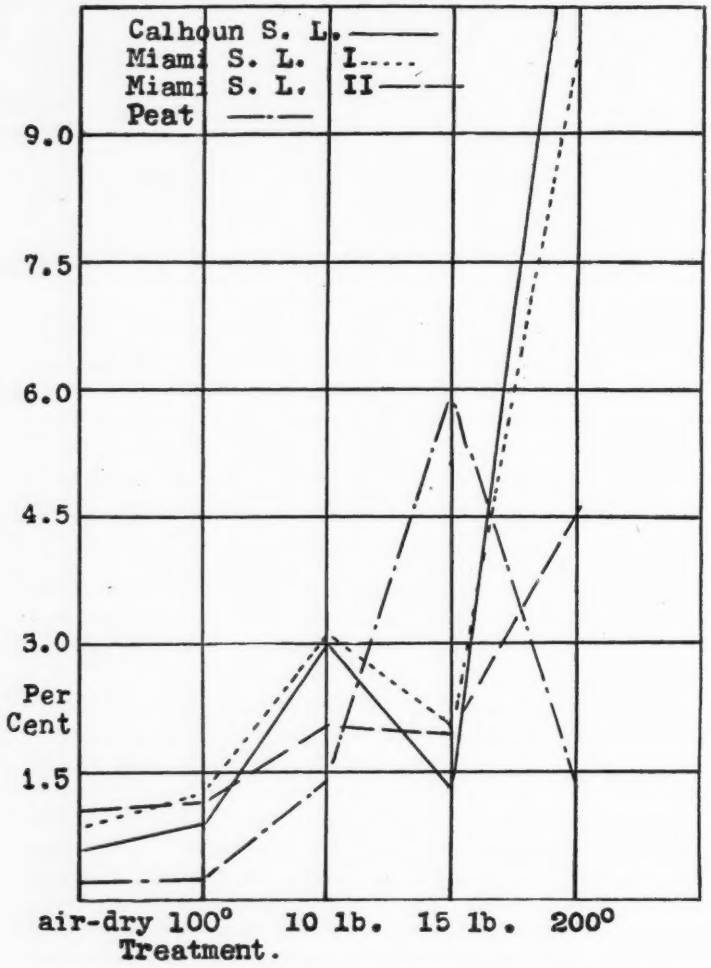

FIG. 1. DIAGRAM SHOWING EFFECT OF HEAT ON AMMONIA IN THE SOILS STUDIED

At 200° the Calhoun soil and the Miami soil I, showed an increase and the other soils a decrease. The two soils that had an increase in amino acid nitrogen also showed the greatest increase in ammonia. Another interesting point is the slight change in amino acid nitrogen shown by the peat soil.

Figure 3 shows the results for the nitrate nitrogen. Perhaps the most interesting thing shown by the figure is that the curves for the various soils are arranged in the order of the per cent of total organic matter present in the

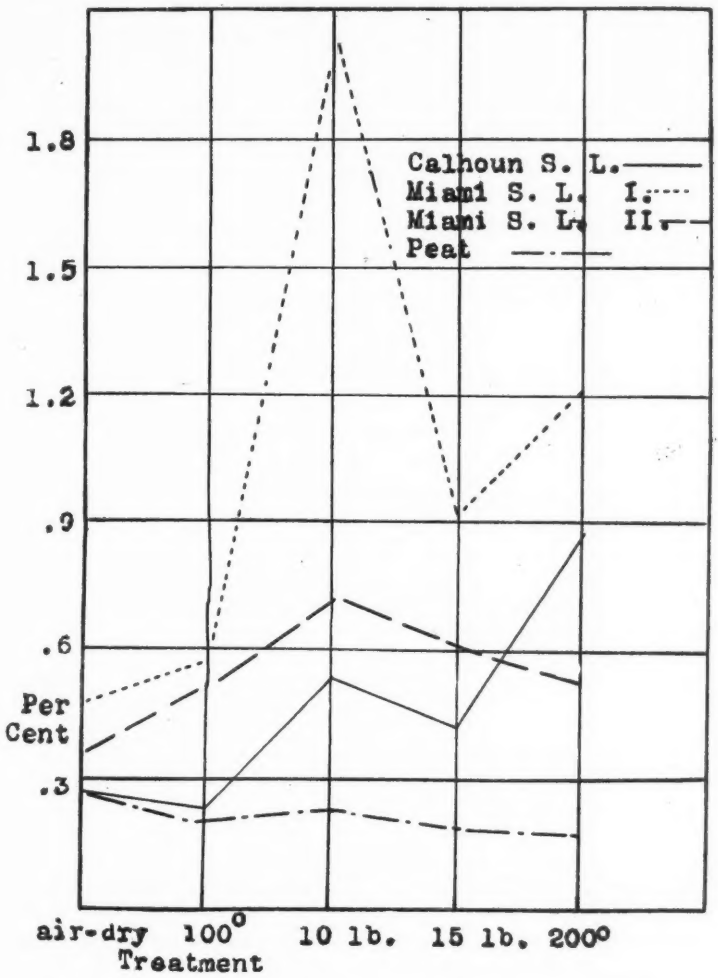

FIG. 2. DIAGRAM SHOWING EFFECT OF HEAT ON AMMONIA ACIDS IN THE SOILS STUDIED

soils. The peat soil, containing the highest amount, being at the bottom and the Miami silt loam soil, containing the least, at the top. Heat at 100° caused a decrease in nitrates in all the soils but the Calhoun silt loam. Ten pounds and 15 pounds in the autoclave caused an increase in the amounts of

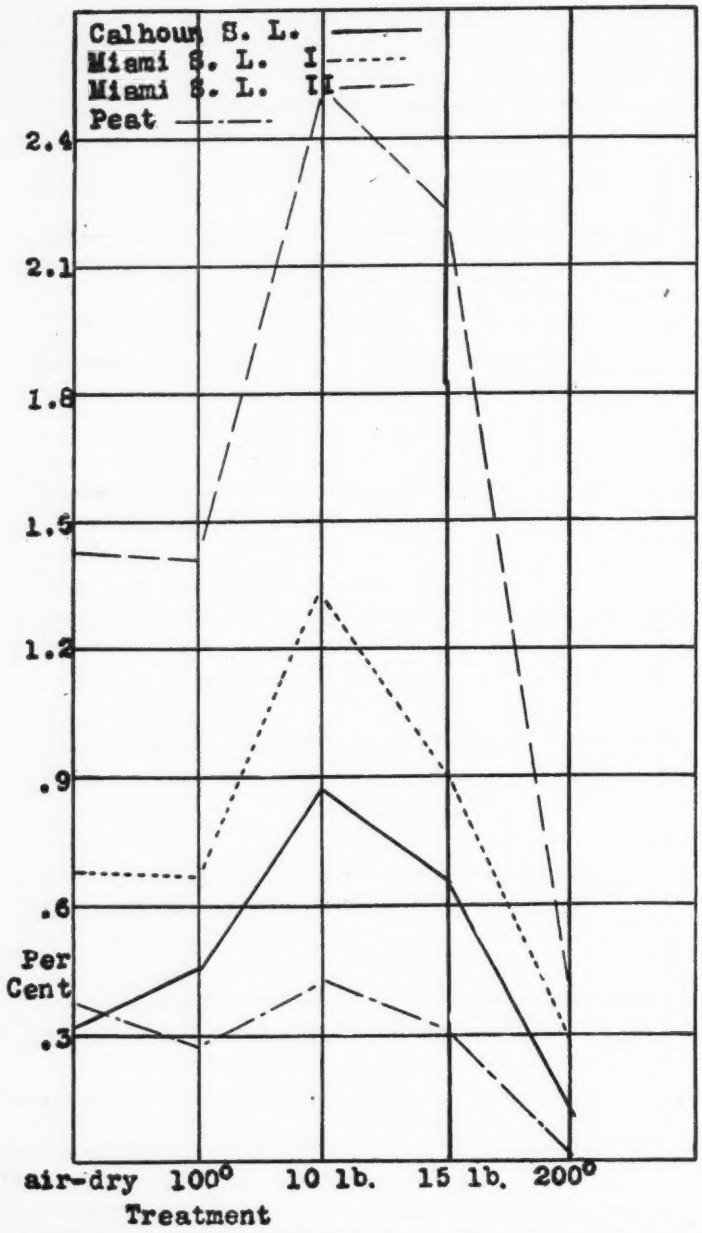

FIG. 3. DIAGRAM SHOWING EFFECT OF HEAT ON NITRATE NITROGEN IN THE SOILS STUDIED

nitrates in all the soils, the 10 pounds a greater increase than the 15 pounds. This is contrary to the findings of Lyon and Bizzell (7, 8) who found that nitrates were greatly reduced by heat at 15 pounds for 2 hours. At 200° for 2 hours it is seen from the figure that the nitrates have been greatly reduced. As pointed out earlier in this paper, this decrease is due to volatilization of nitric acid or reduction of the nitrates by the organic matter.

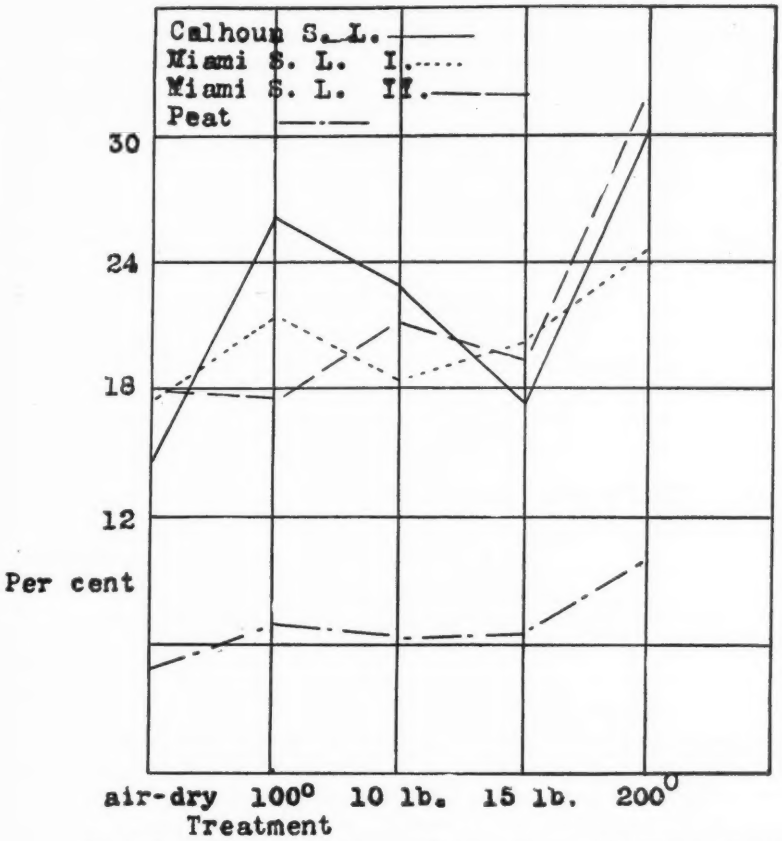

FIG. 4. DIAGRAM SHOWING EFFECT OF HEAT ON SOLUBLE NON-PROTEIN NITROGEN IN THE SOILS STUDIED

The soluble non-protein nitrogen results are shown in figure 4. It is seen that the curves do not resemble very closely either the curves for the ammonia or the nitrate results. This is important in that it shows that when we follow the changes of nitrate and ammonia in a soil we have only half-truths, for while these two substances are perhaps the most important nitrogenous constituents of the soil in that they are used more than any other

compounds as plant nutrients, yet our results show that there is a rather large class of soluble nitrogenous compounds other than the nitrates and ammonia whose transformation does not follow the latter two compounds. In this connection, the results for the soluble non-protein nitrogen for both the incubated and unincubated peat soils are particularly interesting. It is observed that in some cases the sum of the nitrate, amino acid and ammonia nitrogen is nearly equal to, and in one case exceeds, the total soluble non-protein nitrogen. In this one case noted no doubt there must be some error of analysis involved. One source of error in the determination of the soluble non-protein nitrogen is a possibility of the loss of ammonia from the 1½ per cent sodium hydroxide used for the extractive agent. This would be particularly true for these treated soils which contain such large amounts of ammonia.

SUMMARY AND CONCLUSIONS

The following conclusions may be drawn from the soils receiving simply the respective heat treatments.

1. The amount of ammonia was increased by all the heat treatments, the higher temperatures to which the soils were heated giving in general greater increases. At 10 pounds the increase was greater than at 15 pounds, the reason being the longer time the soils were heated at 10 pounds.

The peat soil heated to 200°C. contained less ammonia than the soil heated at any other temperature, the reason being the excessive volatilization of ammonia at that temperature.

2. The amino acid nitrogen results in general followed those for ammonia nitrogen. The peat soil again is an exception, not much of any change being brought about by any of the treatments.

3. Not much change was brought about in the nitrate content by dry heat at 100°C., 10 pounds in the autoclave for 9 hours increased the amount considerably in every soil. Fifteen pounds for 3 hours caused a somewhat less increase than the 10-pound treatment. A temperature of 200°C. caused almost the total disappearance of all nitrates.

4. All the heat treatments caused an increase in soluble non-protein nitrogen. The least change was brought about in the peat soil. This is probably in part due to the fact that considerable amounts of ammonia escaped from the soil at the higher temperature treatments.

The following conclusions may be drawn from the results for the soils heated to 10 pounds in the autoclave for 9 hours and the soil heated to 200°C. for 2 hours, both later inoculated and incubated as stated in the first part of this paper.

1. In the three mineral soils heated to 10 pounds for 9 hours and with 10 weeks' incubation after inoculation with fresh soil, there was an increase of ammonia. Twenty weeks' incubation likewise caused an increase in two

cases, though not such an increase as the 10-week period. Twenty weeks' incubation of the Calhoun soil caused a slight decrease in ammonia. The amount of ammonia in the peat soil after the two different heat treatments was not greatly influenced by either incubation periods.

2. The changes in the amino acid content of the soils after incubation were in general as follows. Ten weeks' incubation caused a decrease in amino nitrogen. There was always more amino nitrogen at the end of 20 weeks than at the end of ten. In two of the soils there was more amino nitrogen at the end of the 20 weeks than there was at the beginning.

3. Nitrates were invariably increased by the two incubation periods, the longer period usually giving the greater increase.

4. The amount of non-protein nitrogen was not changed very much by incubation of the mineral soils which had been heated to 10 pounds. In the peat soil 20 weeks decidedly lessened the amount of this group of compounds. In the mineral soils heated to 200°C. there was always an increase in soluble non-protein nitrogen after incubation. Here also the peat soil showed a decrease for both incubation periods.

REFERENCES

(1) BURGESS, P. S. 1913 The aluminum reduction method as applied to the determination of nitrates in "Alkali" soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, pp. 51–62.

(2) DEHERAIN, P. P., AND DEMOUSSY, M. E. 1896 Sur l'oxydation de la matière organique du sol. *In* Ann. Agron., t. 22, p. 306–337.

(3) GREENWALD, I. 1915 The estimation of non-protein nitrogen in blood. *In* Jour. Biol. Chem., v. 21, p. 61–63.

(4) KELLEY, W. P., AND MCGEORGE, M. 1913 The effect of heat on Hawaiian soils. *In* Hawaii Agr. Exp. Sta. Bul. 30, p. 37.

(5) KOBER, P. A., AND SUGIURA, K. 1915 A micro-chemical method for the determination of alpha and beta amino acids and certain derivatives. *In* Jour. Amer. Chem. Soc., v. 37, p. 1547–1584.

(6) KOPELOFF, N., AND COLEMAN, D. A. 1917 A review of investigations in soil protozoa and soil sterilization. *In* Soil Sci., v. 3, p. 197–271.

(8) LYON, T. L., AND BIZZELL, J. A. 1913 Water-soluble material in soils sterilized and reinoculated. N. Y. Agr. Exp. Sta. (Cornell) Bul. 326.

(7) LYON, T. L., AND BIZZELL, J. A. 1910 Effect of steam sterilization on the water-soluble matter in soils. N. Y. Agr. Exp. Sta. (Cornell) Bul. 275, p. 130–155.

(9) PICKERING, S. U. 1908 The action of heat and antiseptics on plant growth. *In* Jour. Agr. Sci., v. 3, p. 32–54.

(10) PICKERING, S. U. 1908 Studies on germination and plant growth. *In* Jour. Agr. Sci., v. 2, p. 411–434.

(11) POTTER, R. S., AND SNYDER, R. S. 1915 The determination of ammonia in soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 221–226.

(12) POTTER, R. S., AND SNYDER, R. S. 1915 The amino acid nitrogen of soil. *In* Jour. Indus. Engin. Chem., v. 7, p. 1049–1055.

(13) POTTER, R. S., AND SNYDER, R. S. 1916 The soluble non-protein nitrogen of soil. *In* Jour. Agr. Research, v. 6, p. 61–64.

(14) RICHTER, L. 1896 Uber die Verandernungen welche der Boden durch das Sterilisieren erleidet. *In* Landw. Vers. Sta., Bd. 47, p. 269–274.

(15) RUSSEL, E. J., AND HUTCHINSON, H. B. 1909 The effect of partial sterilization of soil on the production of plant food. · *In* Jour. Agr. Sci., v. 3, p. 111–144.
(16) RUSSEL, E. J., AND HUTCHINSON, H. B. 1913 The effect of partial sterilization of soil on the production of plant food. *In* Jour. Agr. Sci., v. 5, p. 152–221.
(17) SCHREINER, O., AND LATHROP, E. C. 1912 The chemistry of steam-heated soils, U. S. Dept. of Agr. Bur. Soils Bul. 89.

DETERMINATION OF LIME AND PHOSPHORIC ACID IN PEAT SOILS: COMPARISON OF JÖNKÖPING WITH BREMEN METHOD[1]

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Recieved for Publication December 1, 1917

INTRODUCTION

In this country the analysis of peat soils has, up to the present time received little attention, as is well illustrated by the failure of the Association of Official Agricultural Chemists to recognize the need of any special methods for the analysis of these as distinct from the mineral or ordinary soils (1, p. 17–29). The Germans have long since recognized such a difference, for the former (4, p. 111–128) prescribing methods quite distinct from those employed with the latter (4, p. 1–110).

During the past 30 to 40 years, a great number of peats have been analyzed in Germany and Sweden, the number in the latter country reaching, according to von Feilitzen (3, p. 164), a total of 3700 samples as early as 1903. This work was begun at the Bremen station and the special method developed there was followed in the Jönköping laboratory of the Swedish Peat Land Cultivation Society for the first few years after it was established in 1886. But another method for the determination of the mineral constitutents of these soils was soon developed and this has since been used exclusively there. We shall refer to this as the Jönköping method.

The only previous comparison of the two methods reported is one from the Jönköping laboratory in 1892 (2, p. 452–457). As a large number of peat soils are being analyzed in this laboratory it appeared desirable to make a further comparison, especially as a recent study by one of us (6) caused us to suspect that the iron content might have an important influence upon the amount of phosphoric acid recoverable by the Jönköping method.

Bremen method

According to the Bremen method (4, p. 118–119), used in general by the German peat experimental stations, a sample of peat is incinerated and the ash extracted with aqua regia. In a hydrochloric acid solution of the material rendered soluble in this manner the percentages of lime and phosphoric acid are determined by the usual procedure.

[1] Published with the approval of the Director as Paper No. 98, of the Journal Series of the Minnesota Agricultural Experiment Station.

Jönköping method

This method has been employed by the Swedish Peat Land Cultivation Society for the analysis of all peat soils since 1890 (3, p. 164). It consists (5) in digesting at room temperature a quantity of air-dried soil equivalent to about 70 gm. of water-free material with 700 cc. of 12 per cent hydrochloric acid for 48 hours. At the end of this time the extract is filtered twice, first through linen and then through filter paper and from the solution thus obtained 50-cc. portions (5 gm.) are taken for the determination of lime and phosphoric acid as well as the oxides of iron and aluminum.

Lime (5. p. 22). A 50-cc. portion of the acid extract is evaporated to dryness and the residue ignited over a Bunsen burner, at first gently, more strongly toward the end, but avoiding any intense ignition. As soon as the contents of the dish have started to glow it is covered with a thin asbestos disc and the ignition continued for three-quarters of an hour, after which the crust formed is broken up with a glass rod and the ignition continued half an hour longer, or until no carbon is to be observed on the sides of the dish or in the ignited mass. As soon as it is free of carbon the residue is pulverized by means of a heavy glass rod or agate pestle, treated with water and a pinch of sal ammoniac, and the mixture digested on the water bath for an hour with occasional stirring. At the end of this time the liquid is filtered off, the residue extracted twice with boiling water, and finally transferred to a filter and washed. The lime is determined in the filtrate, using the permanganate method.

Phosphoric acid (5, p. 26). Another 50–cc. portion of the acid extract is evaporated and incinerated as described above, the residue extracted with hydrochloric acid, filtered, evaporated to dryness and finally taken up with 25 per cent nitric acid. The phosphorus in this solution is determined volumetrically, a normal solution of sodium hydroxide being used as a solvent for the yellow precipitate, and the excess titrated with normal nitric acid, with the factor 1 cc. of normal sodium hydroxide = 0.00315 gm. P_2O_5.

DATA OBTAINED BY C. VON FEILITZEN

There appears to be only one publication that deals with the relative amounts of the various constituents obtained by the above-mentioned methods. Von Feilitzen (2, p. 449–457) in 1892 reported a comparison in the case of 11 Swedish peats and mucks. The portions of his data bearing on the present subject have been assembled in table 1.

It is to be observed that the amount of lime obtained by the two methods is, for practical purposes, about the same, it being slightly the lower with the Jönköping method, but that only from one-half to less than one-third as much phosphoric acid is obtained by the latter method. While the amounts of the oxides of iron and aluminum do not differ widely, if the three muck

soils, the last in the list, are left out of the comparison, the Bremen method gives the larger amount in every case.

EXPERIMENTAL

Eight samples of peat soil, varying in lime content from 0.6 per cent to 3.9 per cent, were used in this study. The composition, depth and source of each sample are shown in table 2.

TABLE 1

Comparison of the Bremen (B) and Jönköping (J) methods for the analysis of peat and muck soils, as shown by data obtained by von Feilitzen

SAMPLE	DEPTH	VOLATILE MATTER	Fe_2O_3	Al_2O_3	CaO		P_2O_5	
			B	J	B	J	B	J
	cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Highmoor, Flahult	0–30	98.05	0.24	0.16	0.21	0.16	0.11	0.05
	30–60	98.60	0.29	0.22	0.19	0.21	0.06	0.03
	60–90	98.59	0.30	0.30	0.17	0.18	0.06	0.02
	90–120	98.58	0.46	0.39	0.13	0.13	0.07	0.02
	120–150	98.63	0.71	0.57	0.11	0.10	0.07	0.01
Lowmoors, Flahult	0– 20	88.85	3.09	2.74	0.04	0.08	0.15	0.07
Martebo myr	0– 20	83.38	2.00	1.40	8.48	8.64	0.12	0.04
Vårgårda	0– 20	70.71	1.45	0.78	1.35	1.17	0.10	0.03
Vargsäter	0– 20	46.32	10.12	5.12	2.89	2.71	0.25	0.10
Skeppsholmen	0– 20	25.59	7.39	4.57	0.61	0.28	0.30	0.13
Högs gård	0– 20	21.02	8.52	3.92	0.59	0.22	0.22	0.10

TABLE 2

Comparison of the Bremen (B) and the Jönköping (J) methods in the analysis of Minnesota peat soils

SAMPLE	SOURCE OF SAMPLE, COUNTY	DEPTH	VOLATILE MATTER	$Fe_2O_3+Al_2O_3$ J	CaO		P_2O_5	
					B	J	B	J
		inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Anoka	0–8	74.3	5.66	3.91	3.35	0.55	0.34
2	Anoka	0–8	77.8	4.21	3.01	2.60	0.42	0.20
3	Anoka	0–8	77.4	3.20	1.36	1.10	0.30	0.10
4	Anoka	0–8	83.4	3.95	2.25	1.94	0.33	0.10
5	Ramsey	0–8	83.8	1.22	1.24	0.94	0.32	0.11
6	Itasca	9–16	90.7	1.55	0.84	0.60	0.20	0.03
7	Blue Earth	9–16	78.0	1.36	2.79	2.50	0.23	0.07
8	Itasca	0–8	89.5	1.10	0.80	0.55	0.25	0.09

In order to be able to compute the amount of the moist peat equivalent to 70 gm. of dry material, as specified in the Jönköping method, duplicate moisture determinations were made by drying small samples for 24 hours in a water oven. In adding the 12 per cent hydrochloric acid the water in the peat was taken into consideration and allowance made for this, enough con-

centrated hydrochloric acid being added after the bulk of the 12 per cent acid had been put on the sample to bring the final strength of the hydrochloric acid up to the required 12 per cent. The digestion was carried out in liter bottles with frequent shaking. At the end of the 48-hour period the supernatant liquid was filtered off and the analyses made as described above.

In the Bremen method 5-gm. samples were weighed out in silica dishes and ignited, at first very gently but finally in the muffle. The ash thus obtained was evaporated with 25 cc. of aqua regia to complete dryness twice and then taken up with hydrochloric acid, diluted to 500 cc. with water and an aliquot part of this taken for each determination.

The eight samples were analyzed by both of the above-described methods. The results are shown in table 2. The same general observations made in regard to the work reported by von Feilitzen (table 1) may be applied to these. While the amount of lime obtained by the Bremen method is in every case greater than that obtained by the Jönköping, the difference in amount is not so great but that the percentage of this constituent found adequate for plant growth by the former should also prove adequate when the determination is made by the latter.

The phosphoric acid obtained by the Bremen method is from two to three times greater than that obtained by the Jönköping. Von Feilitzen (3, p. 160–162) has already pointed out that the difference in the amount of this constituent obtained by the two methods constituted their chief difference, claiming that the smaller amount obtained more truly represented that actually available for plant growth since much of the phosphoric acid found when a peat is ashed is not available for plant use.

Completeness of recovery of lime and phosphoric acid

The completeness of recovery of the lime and phosphoric acid in the acid extract obtained by the Jönköping method was tested. The data are reported in table 3. It is to be seen that not the whole of the lime is recovered, in most cases sufficient being found in the residue to make the total amount extracted by the acid equal to that found by the Bremen method. As a measure of precaution we had extracted the residue five times with hot water instead of only twice, as is done in the Jönköping laboratory. So the shortage cannot be attributed to insufficient washing of the residue preceding its removal from the dish to the filter. It is of interest to note that the amount found in the residue varies directly with the percentage of oxide of iron. In general the higher the percentage of this constituent the larger was the amount of lime found in the residue. There is no definite relation between the amount of lime in the residue and the amount of oxide of aluminum.

Practically the whole of the phosphoric acid extracted by the 12 per cent hydrochloric acid is recovered, only a negligible quantity for the most part

being left in the residue. It will be seen that in the cases where the larger amounts are found in the residue the sample carries a comparatively high percentage of iron oxide. One of us has already shown (6, p. 306–307) that phosphoric acid may be held in the residue when this has been heated too strongly, as a result of the formation of difficulty-soluble phosphates of iron and aluminum. There appears to be no definite relation between the amount of oxide of iron present and the amount of phosphoric acid extracted.

SUMMARY

1. Two methods for the determination of lime and phosphoric acid in peat soils are compared, viz., the Bremen, in which the sample is incinerated and the ash extracted with aqua regia, and the Jönköping, in which an air-dry sample is extracted with 12 per cent hydrochloric acid.

TABLE 3

Analysis of Minnesota peat soils by the Jönköping method, showing the completeness of recovery of lime and phosphoric acid from the acid extract

SAMPLE	Al_2O_3	Fe_2O_3	LIME (CaO)			PHOSPHORIC ACID (P_2O_5)		
			Original extract	Residue from extract	Total amount extracted	Original extract	Residue from extract	Total amount extracted
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1.89	3.77	3.35	0.67	4.02	0.34	0.020	0.360
2	1.69	2.52	2.60	0.37	2.96	0.20	0.014	0.214
3	1.35	1.85	1.10	0.28	1.38	0.10	0.004	0.104
4	2.38	1.57	1.94	0.21	2.15	0.10	0.003	0.103
5	0.79	0.43	0.94	0.18	1.12	0.11	0.002	0.112
6	1.18	0.37	0.60	0.05	0.65	0.03	0.003	0.033
7	1.03	0.33	2.50	0.13	2.63	0.07	0.005	0.075
8	0.80	0.30	0.55	0.14	0.69	0.09	0.002	0.092

2. With the former, larger amounts of iron and aluminum oxides, lime and phosphoric acid are obtained, the difference with the first three constituents being small, but that of the last very large.

3. The smaller amount of lime found by the Jönköping method is due to part of that extracted being rendered insoluble in water in the course of the analysis and hence not included in the final precipitation. This unrecovered portion varies directly with the amount of iron present. Practically all of the phosphoric acid is recovered from the acid extract.

REFERENCES

(1) Association of Official Agricultural Chemists 1916 Methods of analysis. *In* Jour. Assoc. Off. Agri. Chem., v. 1, no. 4, pt. 2, p. 17–29.

(2) VON FEILITZEN, CARL 1892 Forvaltningstutskottets berättelse till revisorerna öfver räkenskaper och förwaltning 1891. *In* Svenska Mosskulturför. Tidskr., v. 6, no. 5, p. 442–481.

(3) VON FEILITZEN, HJALMER 1910 Über die chemische analyse des Moorbodens bei der Bewertung für Kulturzwecke. *In* Verhandlungen der Zweiten Internationalen Agrogeologenkonferenz, p. 160–168. Stockholm.

(4) KÖNIG, J. 1911 Untersuchung Landwirtschaftlich und Gewerblich Wichtiger Stoffe. Ed. 4, 1226 p. 426 fig. Berlin.

(5) LUGNER, IVAR 1912 Metod för undersökning af torfjord för kulturändamal, anväud vid Svenska Mosskulturföreningens kemiska laboratorium. *In* Berättelse öfver Verksamheten vid Svenska Mosskulturföreningens Kemiska Laboratorium år 1911, p. 22–26.

(6) ROST, CLAYTON O. 1917 The determination of soil phosphorus. *In* Soil Sci., v. 4, p. 295–311.

POTASSIUM REQUIREMENTS OF BACTERIA[1]

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Received for publication July 1, 1917

INTRODUCTION

The knowledge that plants require certain mineral constituents in order to continue their life processes, dates back to the time of Liebig (6). It was, however, not definitely known until 1870 just what these essential salts are. In that year Nobbe (7) and his associates, through careful investigation, demonstrated to what extent plant activities would continue in the absence of the various elements. The proof that plants of the lower order require the same essential mineral constituents is now quite well established.

The literature relating to the influence of certain inorganic salts on biological activities is voluminous. Some of the researches on the subject have been referred to in another paper by the writer (2). In the data set forth by Gerlach and Vogel (1) in 1903, and by Vogel (10) in 1912, these investigators demonstrated the extent to which microbial activities (fixation of nitrogen by *Azotobacter chroococcum*) take place when certain of the essential elements are not supplied. In a similar way Krzemieniewska (5) in 1910 showed that K, Ca, Mg, P and S were all essential to the development of *Azotobacter*. In these experiments the physical factor, namely, the effect upon the microbial activities in varying the total osmotic concentration of the various solutions when demonstrating the effect of the various cations and anions, was not considered. Also, the antagonism of the various salts was not observed.

Since it has been demonstrated by the respective methods of the above investigators that potassium is one of the essential inorganic cations for bacteria (nitrogen-fixing) it was deemed of scientific importance to verify these results, if possible, by methods in which the physical factors were controlled. Still further, it appeared desirable to ascertain to what extent potassium is a factor. In other words, how much of this element do microorganisms require in order to carry on their life processes?

EFFECT OF VARIATION IN TOTAL CONCENTRATION AND LACK OF POTASSIUM UPON BIOLOGICAL ACTIVITIES

Tottingham (9) and Shive (8), working with plants, and the writer (3), in a previous work with microorganisms, demonstrated to what extent the

[1] Paper read at the meeting of the Society of American Bacteriologists, at Washington, D. C., December 27, 1917.

total concentration (all other factors, as the arrangement of salts, etc., being constant) influences life processes. At a very low concentration (total 0.10 atmosphere) the writer found practically no difference in the formation of ammonia from dialyzed peptone by *Bacillus subtilis* in the various combinations of the three salts. For this reason, and also since a considerable amount of ammonia was formed in all the solutions of the sub-optimal series (total concentration 0.10 atmosphere) and the amounts corresponded very favorably with the amounts formed in the optimal series (total concentration 1.0 atmosphere), this concentration (0.10 atmosphere) was adopted as the most desirable for investigations to ascertain the requirements of microorganisms.

At such a low concentration as 0.10 atmosphere of diffusion tension, keeping the salts in their same proportion, to what extent is it possible to vary the total concentration of the solutions without altering the results? An experiment was performed to ascertain if it is possible to reduce the total concentration from 0.1 to 0.09 atmosphere without materially affecting the ammonia production.

The Gibbs triangle scheme which was successfully employed in previous biological work (3, 4) was used. In this case, however, it was only to make use of the solutions appearing on the left-hand side of the triangle where the potassium sulfate furnishes but one-tenth of the total diffusion tension, and the other two salts are in various proportions. These solutions are indicated as R_1C_1, R_3C_1, R_5C_1, R_7C_1 and R_8C_1.

Three series of each of the solutions, R_1C_1, R_3C_1, etc., were prepared. In series I, the solutions were identical to the corresponding solutions of the sub-optimal series of the previous work (3), in which the total concentration of the three salts added was 0.10 atmosphere. In series II the three salts were all in the same proportion as the corresponding solutions in series I, but the total amount of salt was one-tenth less than in series I. In series III the proportion of the two salts magnesium sulfate and calcium phosphate was the same as in the other two series, the total amount of each the same as in series I, and the total osmotic concentration the same in series II. To test the biological activity in the absence of potash, in the third series potassium sulfate was left out of the combination.

The method of calculating and preparing solutions of known concentration was the same as is given in detail in the previous paper (3). Similarly dialyzed peptone, the equivalent of 100 mgm. of nitrogen, of the same stock solution was used. As before, the solutions were inoculated with a pure culture of *B. subtilis* and incubated for 7 days at 30°C., at the end of which period the ammonia determinations were made. All determinations were made in duplicate or triplicate, the average of which is recorded in table 1. The experimental error was never more than 3.5 per cent.

The results for series I as presented in the table 1 demonstrate that there was the same amount of ammonia formed in the various solutions composed of the different combinations of the three salts. This corroborates the results

previously obtained by the writer. In like manner, the same amount of ammonia was realized in the various solutions of series II. This was also true in the third series. As the results of series I are practically the same as those of series II, we would conclude that reducing the total concentration from 0.10 to 0.09 atmosphere, while keeping the salt proportion the same, under the conditions of the experiment would not cause any difference in the biological activities.

TABLE 1

Effect of different concentrations of various combinations of 3-salt solutions and similar solutions without potassium sulfate upon the formation of ammonia from dialyzed peptone by Bacillus subtilis

SERIES NO.	SOLUTION NO.	SALT PROPORTION	TOTAL CONCENTRATION	AMOUNT OF AMMONIA FORMED
				mgm. N
1	R_1C_1.....	$1MgSO_4$ $1K_2SO_4$ $8Ca(H_2PO_4)_2.2H_2O2H_2O$	0.10	34.0
1	R_3C_1.....	$3MgSO_4$ $1K_2SO_4$ $6Ca(H_2PO_4)_2.2H_2O2H_2O$	0.10	33.2
1	R_5C_1.....	$5MgSO_4$ $1K_2SO_4$ $4Ca(H_2PO_4)_2.2H_2O2H_2O$	0.10	33.5
1	R_7C_1.....	$7MgSO_4$ $1K_2SO_4$ $2Ca(H_2PO_4)_2.2H_2O2H_2O$	0.10	33.6
1	R_8C_1.....	$8MgSO_4$ $1K_2SO_4$ $1Ca(H_2PO_4)_2.2H_2O2H_2O$	0.10	33.2
2	Same as in series I....	Same as in series I.	0.09	33.6*
3	Same as in series I....	Same as in series I, except no K_2SO_4	0.09	9.0
Check (peptone alone)				9.0

* As the results of the various determinations of series II were all practically the same, as was also the case in series III, it is not necessary to repeat all the figures.

In comparing the results of series III with those of the other two series, it is apparent that potassium is the limiting factor. While in series I and II we have the same arrangement of salts but a difference of 0.01 atmosphere in total concentration, the same amounts of ammonia were formed in both cases. Hence the slight variation in the partial concentration of each individual salt and the difference in the total concentration of the solution were

not influencing factors. In series I, the partial diffusion tension furnished by magnesium sulfate and calcium phosphate is the same as in series III, and the two series differ only in the total concentration, which is 0.01 atmosphere less in series III and in the absence of potassium sulfate in the latter. That the slight difference in total concentration does not influence the results is apparent from a comparison of series I and II, hence the difference of 24.5 mgm. of nitrogen produced in the two series must be attributed to the lack of potassium.

Also, the addition of magnesium sulfate and calcium phosphate, with a concentration of 0.09 atmosphere, without applying potassium sulfate, did not increase the ammonia formation over that of the check (peptone alone). A chemical analysis of the dialyzed peptone which was used showed the presence of the following: 0.02 per cent P_2O_5; 0.0044 per cent SO_3, 0.0024 per cent K_2O, 0.0067 per cent CaO, and 0.00234 per cent MgO. Since 5 cc. of the peptone furnishing 100 mgm. of nitrogen was used, 0.1 mgm. of K_2O was added in each determination. As is shown in series III, this amount of potash furnished sufficient potassium to allow the formation of 9.0 mgm. of ammonia.

That the cation K and not the anion SO_3 was the limiting factor, since there was sufficient SO_3 furnished by magnesium sulfate in series III to supply the needs of the organisms, has already been demonstrated in previous experiments. In the sub-optimal series, practically the same amounts of ammonia were formed irrespective of the amounts of the various salts and SO_3.

THE EXTENT OF POTASSIUM REQUIREMENTS BY BACTERIA

With the above data at hand, an experiment was performed to ascertain how much potassium organisms require to fulfil their needs. The total concentration of the solution used varied from 0.10 to 0.09 atmosphere. Four sets of three solutions each were prepared as before. In all cases magnesium sulfate furnished one-tenth and calcium phosphate eight-tenths of the total concentration. In the first set potassium sulfate furnished one-tenth of the concentration, in the second set one-half as much potassium sulfate was added as in the first, in the third one-fifth as much, and in the fourth set no potassium sulfate was added. A set of check determinations were made at the same time.

From the data in table 2 it is evident that the organism used (*B. subtilis*) obtained sufficient potassium from 0.24 mgm. of K_2O in 100 cc. of solution to carry on its maximum activities for 7 days at 30°C. Moreover, additions of two and one-half and five times this amount of potash were not effective, within experimental error, in increasing the ammonia formation. The addition of but 0.24 mgm. of K_2O in set 3 increased the ammonia formation 19.6 mgm. over that in set 4. In comparing set 4 with the check, a slight increase in ammonia formation is evident when magnesium sulfate, calcium phosphate and no potassium sulfate were added. This amount, however, is practically

within experimental error. As is seen from an analysis of the peptone, 0.1 mgm. of potash was supplied with the dialyzed peptone, but this amount was sufficient to cause a production of only 8.7 mgm. of NH_3 expressed as nitrogen even after the other two salts were added. However, when a very small amount of this salt is added, a very great increase in activity is realized. Such minute amounts suffice probably because these elements are utilized again and again during the various successive cycles of the organisms.

TABLE 2

Potassium requirements for microorganisms as determined by the amounts of ammonia formed from dialyzed peptone by Bacillus subtilis

SET NO.	SALT PROPORTION	ACTUAL AMOUNTS OF K_2O ADDED	TOTAL CONCENTRATION OF SALT SOLUTION	AMMONIA FORMED	AMMONIA	
					Increase due to salts added	Increase due to K_2O
		mgm.	*atm.*	*mgm. N*	*mgm. N*	*mgm. N*
1	$1MgSO_4$ $1K_2SO_4$ $8Ca(H_2PO_4)_2.2H_2O$	1.25	0.100	29.9	23.6	21.2
2	$1MgSO_4$ $0.5K_2SO_4$ $8Ca(H_2PO_4)_2.2H_2O$	0.62	0.095	30.3	24.0	21.6
3	$1MgSO_4$ $0.2K_2SO_4$ $8Ca(H_2PO_4)_2.2H_2O$	0.24	0.092	28.3	22.0	19.6
4	$1MgSO_4$ $0K_2SO_4$ $8Ca(H_2PO_4)_2.2H_2O$	0.00	0.090	8.7	2.4	
Check (peptone alone)				6.3		

SUMMARY

With the physical conditions controlled, the results of the experiments here recorded indicate that:

1. By keeping the salts in the same proportion, the total concentration of a 3-salt solution can be reduced from 0.10 to 0.09 atmosphere without influencing the extent of ammonia formation from dialyzed peptone by *Bacillus subtilis*.

2. Potassium is essential to the development and activities of *Bacillus subtilis*.

3. If sufficient potassium for microorganisms is not present, magnesium sulfate and calcium phosphate will not influence the activities of (cannot be utilized by) *Bacillus subtilis*.

4. Supplying 0.24 mgm. of K_2O in addition to 0.1 mgm. already supplied in the dialyzed peptone in 100 cc. of solution, furnished sufficient potassium for the maximum development and activity of *Bacillus subtilis*. Applying two and one-half or five times this amount of potash did not increase the activity of the bacteria over that produced with 0.24 mgm.

REFERENCES

(1) GERLACH, M., AND VOGEL, I. 1903 Weitere Versuche mit Stickstoff-bindenden Bakterien. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 10, p. 636–643.

(2) KOCH, GEORGE P. 1917 The effect of potassium salts upon biological activities. A thesis submitted to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1917.

(3) KOCH, GEORGE P. 1917 Physiological balance in nutrient media for bacteria. Author's manuscript: not yet in print.

(4) KOCH, GEORGE P. 1917 Effect of various salts upon the formation of ammonia by soil organisms. Paper presented at the meeting of the Biological-Chemical Section of the American Chemical Society at Kansas City, Mo., April 10, 1917.

(5) KRZEMIEMEWSKA, HELEN 1910 Influence of the mineral constituents of nutritive solutions on the development of *Azotobacter*. *In* Bul. Internat. Acad. Sci. Cracovie, Cl. Sci. Math. et Nat., Ser. B., p. 376–413.

(6) LIEBIG, JUSTUS 1840 Chemistry in its Application to Agriculture and Physiology. Editions 1 and 2 (1840–1841).

(7) NOBBE, F., SCHRÖDER, J. AND ERDMANN, R. 1870 Über die organische Leistung des Kalium in der Pflanze. *In* Landw. Vers. Stat., Bd. 13, pp. 321–323.

(8) SHIVE, JOHN W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, no. 7, p. 327–397.

(9) TOTTINGHAM, W. E. 1914 Quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Res., v. 1, p. 133–245.

(10) VOGEL, I. 1912 Untersuchungen über das Kalibedürfries von Azotobacter. *In* Centbl. Bakt. (etc.), Bd. 32, p. 411–420.

STUDIES ON THE CORRELATION BETWEEN THE PRODUCTION OF CARBON DIOXIDE AND THE ACCUMULATION OF AMMONIA BY SOIL ORGANISMS

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Received for Publication October 15, 1917

INTRODUCTION

The decomposition of organic matter in the soil being generally a biological process, various indexes of biological activity have been used to measure it, the more common of which have been plate counting, ammonification and nitrification. Wollny (14), Lemmerman and his associates (4), Stoklasa and Earnest (10), van Suchtelen (12), and others have shown that the evolution of carbon dioxide is an excellent index of the decomposition of soil organic matter. Later work has been done by Fred and Hart (2) and by Potter and Snyder (9) concerning the influnce of sulfates, phosphates and lime on carbon-dioxide production. Ammonification studies have been numerous but a survey of the literature yields little on the relationship between ammonia accumulation and carbon-dioxide production.

Discrepancies have been observed between an apparent increased biological activity in soils and an increased ammonia accumulation or vice-versa. Lipman and his associates (6) found that the addition of soluble carbohydrates decreased the accumulation of ammonia, and, in later work (7), of nitrates also, the production of carbon dioxide, however, being increased. It is evident that ammonia accumulation in itself can seldom be taken as a criterion of the intensity of action taking place. While ammonia is readily assimilated by most microorganisms and may therefore disappear as soon as liberated, the carbon dioxide produced is not used again by the same or by other species. Since carbon dioxide is not assimilated it becomes very nearly an absolute index of biological activity, with due regard to possible chemical formation under certain conditions.

In view of the fact that ammonia determinations are much more easily made and because much valuable ammonification data have been obtained, an attempt is being made to learn more of the relationship between the assimilation of ammonia and the actual activity as shown by the production of carbon dioxide. There must be an optimum ratio between these two factors necessary for the most favorable soil conditions.

Considerable attention is given here to the activity of pure cultures of some of the more common species of soil organism, both bacteria and fungi. Work with a fresh soil or soil infusions involves so many factors and species that it is difficult to interpret the results obtained, or at least to know the real effect or effects resulting from changing some of the conditions of the experiment. Results obtained with pure cultures and with mixtures of the same in the natural soil medium, except as it is changed by sterilization, ought to be of value in interpreting the action of the natural flora and to aid, ultimately, in the management and improvement of soils so that they will yield the greatest possible returns without deterioration.

Without at this time giving a more complete historical review there is here given a description of methods and of the apparatus, together with some of the data so far obtained.

THE APPARATUS AND ITS MANIPULATION

Some of the chief difficulties in obtaining a satisfactory apparatus for the determination of carbon dioxide evolved biologically, have been the lack of a source of slow, though continuous aspiration, too high a vacuum within certain of the parts, incomplete removal of the carbon dioxide from the air entering, incomplete absorption of the same gas produced, leakage, clogging of tubes, back suction and inconvenience in manipulation. It is believed that the apparatus used in this work does away with many of these defects although it may yet be much improved. Figure 1 gives a diagram of the different parts, and a photograph of a few of the units is given in plate 1. Air enters the bottle *A* containing strong sodium or potassium hydroxide and passes through the tower of glass beads inserted into the bottle. Most of the carbon dioxide and much of the moisture is removed in this tower which lies at an angle which may be varied to suit conditions. The air then passes through the soda-lime tube *B* for the more complete removal of carbon dioxide. From thence it enters the distributing bottle *C*, bubbling through a dilute acid, in this case 10 per cent sulfuric acid, before entering the bell-jar *D*. The bell-jar stands on a pine or cypress board which is prepared by painting on both sides with hot paraffin wax. A hole large enough for the insertion of a no. 5 2-holed rubber stopper is bored beneath each bell-jar which is made air-tight against the paraffined board by means of a brush and molten paraffin. The paraffin thus used was softened somewhat by melting it up with one-third its weight of paraffin oil. The air, drawn from the bottom of the bell-jar, passes in through the side neck of the suction flask and up through the barium hydroxide bead tower *E*, which removes the carbon dioxide produced by the organisms in flask *F*. From thence the line leads to the suction pump *G* which is fed by a siphon from the constant-level water tank *H*.

In the apparatus as used, a bell-jar, tower and distributing bottle are connected up in sets of six, each leading back to one soda-lime tube and ahead

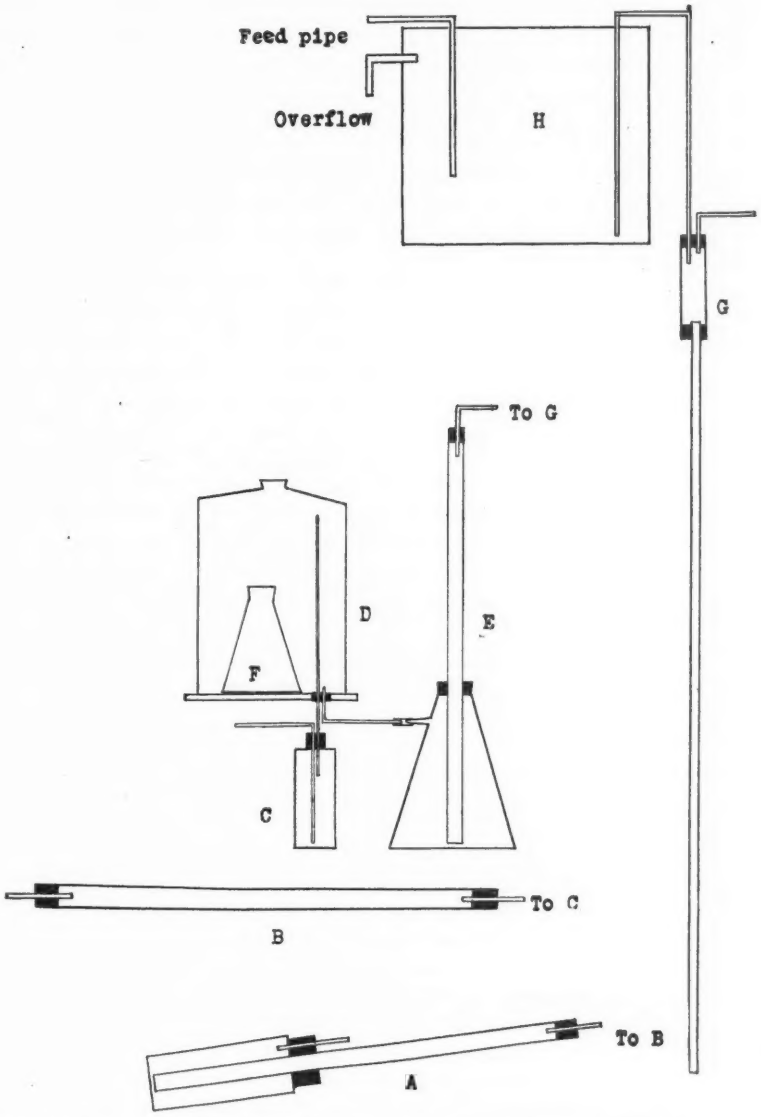


FIG. 1. A DIAGRAM OF THE APPARATUS USED TO DETERMINE CARBON DIOXIDE EVOLVED FROM SOIL[1]

[1] The principle involved in the pump was obtained from apparatus used by Dr. T. J. Headlee, entomologist of the New Jersey Agricultural Experiment Station.

to one suction pump. Each bell-jar and tower is in a separate line, however, diffusion of carbon dioxide being prevented by the acid solutions of the distributing bottles *C*. The suction pump *G* as finally constructed produced a very constant, continuous suction and required but little water for its operation. The tube leading from it being larger than the intake tube, bubbles of air are drawn down by the water in its descent. The pump itself consists of an ordinary thick-walled test-tube, fitted with stoppers at top and bottom, the closed end of the tube being broken off. The length of the drain tube largely determines the suction power and it was found that a tube 4 to 5 feet long was sufficient.

Clogging of the air inlet by the formation of crystals of sodium carbonate in the strong sodium hydroxide solution was prevented by using a bead tower *A* as shown, the opening at the bottom of the tower being 24 mm. in diameter. This tower was put in a slanting position to permit a slow, constant intake of air and to prevent as much of a vacuum as possible within the bell-jars.

The barium hydroxide tower which is 24 inches high is a modification of one described by Truog (11). The side-necked suction flask is of the type ordinarily used for Gooch crucible work. The barium hydroxide solution, used to absorb the carbon dioxide liberated, was made by adding 40 gm. of $Ba(OH)_28H_2O$ per liter of distilled water. The solution was siphoned off after standing over night and kept in a bottle protected from the carbon dioxide of the air, delivery being made through an automatic burette. The oxalic acid solution, used to titrate the excess of barium hydroxide, contained 8.6 gm. of the acid per liter. Both solutions were standardized in terms of milligrams of carbon dioxide per cubic centimeter. The procedure consisted of first adding the desired amount of barium hydroxide solution to the side-necked flask together with a few drops of phenolphthalein as an indicator and enough carbon-dioxide-free water to cause the liquid to rise about half way up in the bead tower when aspirating through the apparatus. When ready to make a determination the tower was partly withdrawn and washed free from barium hydroxide with carbon-dioxide-free water, the glass beads remaining in place because of the cloth gauze stretched over the bottom of the tube. The flasks were kept stoppered until titrated with oxalic acid, the residual barium hydroxide being thus determined. This method, although expeditious, results in some carbon-dioxide absorption from the air. Tests were conducted by aspirating for the customary periods with sterile flasks in the bell-jars, and the average blank thus obtained was subtracted from each determination.

The construction of the apparatus is such as absolutely to prevent any back suction of solution through the various units leading to the bell-jars. The amount of air aspirated was controlled with screw pinch cocks and was slow but continuous. Previous to a determination the air was allowed to circulate more rapidly for an hour. In view of the unquestioned value of carbon-dioxide determinations, it is hoped that a set of apparatus may finally

be perfected that will become as permanent a piece of laboratory equipment as an ammonia or a nitrogen still. Instead of bell-jars, for instance, metal jars, closed at the bottom and fitted with air-tight, removable covers, might be used.

METHODS

The bacteria used were grown on bouillon agar slants made in square bottles about 4 cm. wide and 10 cm. high. These were incubated at 28°C. for from 4 to 6 days. When ready to use, 100 cc. of a sterile 0.6 per cent salt solution was added to each bottle. A sterile rubber stopper was then inserted, and a suspension obtained by shaking gently. Erlenmeyer flasks of 250 cc. capacity, containing 70 gm. of soil were sterilized at 15 pounds steam pressure for 20 minutes and inoculated with 3 cc. of the suspensions. When mixtures of pure cultures were used for inoculating, these were obtained by pipetting equal amounts of the desired suspensions into a sterile flask from which 3-cc portions were drawn after gently shaking the mixture. This method of mixing reduces the total number of a given species but the inoculation was so heavy, at any rate, that this decrease was considered negligible.

When fungus cultures were employed the procedure was similar, except that a longer incubation of the inoculating material was necessary to secure abundant sporulation. The medium used consisted of 0.25 gm. of magnesium sulfate, 0.25 gm. of di-potassium phosphate, 10 gm. of peptone, 20 gm. of glucose, 15 gm. of agar and 1000 cc. of water. The soil infusions used were obtained from a neutral loam well supplied with organic matter.

The pure cultures employed were carefully tested as to purity and characteristics and were obtained from the stock cultures of the soil department of the New Jersey Agricultural Experiment Station. Several tests made upon flasks of soil which had been inoculated in the above manner showed no contamination with foreign organisms.

The soil was mixed with the organic material and other ingredients added by shaking for 5 minutes in a large bottle. The 70-gm. portions were then weighed out with a balance sensitive to 0.1 gm. The water content was 50 per cent of the maximum as determined by the Hilgard method. Seventy-gram instead of 100-gm. portions were used because a thinner layer with the same surface area was obtained, thus permitting better aeration. In the work done so far a soil classified as Norfolk sandy loam has been used. This contains sand of fine texture and has a maximum water-holding capacity of 32 per cent. It is low in organic matter, containing only 0.02 per cent of nitrogen, and has a lime requirement of 550 pounds of calcium oxide per acre as determined by the Veitch method.

Preliminary tests showed that the ammonia accumulation was about the same whether flasks were incubated without bell-jars or within, through which air was slowly but continuously circulated. The loss of moisture for a 12-day period was 1.2 gm. per flask within the bell-jars and 2 gm. without. Ac-

cordingly, some of the flasks for ammonia determinations were incubated apart from the apparatus during the same period that carbon-dioxide determinations were being made. The ammonia was determined by adding an excess of magnesium oxide and distilling in the usual way.

In all cases checks have been subtracted from the reported amounts of carbon dioxide and of ammonia, which are the averages of duplicate determinations. The inoculated flasks were incubated in a room kept at a temperature of from 21° to 23°C. for a period of 12 days.

TABLE 1

A comparison of duplicate determinations of carbon dioxide production and of ammonia accumulation

ORGANISMS	CO_2 PRODUCTION				NH_3 ACCUMULATION			
	1	2	Average	Per cent of error from mean	1	2	Average	Per cent of error from mean
Norfolk sandy loam + 1 per cent of cottonseed meal								
	mgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	
B. subtilis	134.1	138.8	136.5	3.4	10.38	11.40	10.59	9.5
B. vulgatus	169.1	167.0	168.1	1.3	7.85	8.61	8.23	9.2
B. subtilis + B. vulgatus	215.3	201.2	208.3	6.7	10.38	11.00	10.69	3.0
B. mycoides	60.4	Lost			2.00	2.27	2.19	12.3
B. megatherium	45.3	37.7	41.5	18.3	1.17	1.67	1.42	35.0
B. mycoides	40.7	43.5	41.6	4.1	1.42	1.42	1.42	00.0
B. mycoides + B. megatherium	38.4	39.6	39.0	3.0	2.82	2.32	2.57	2.0
B. mycoides + B. vulgatus	194.2	202.3	198.3	4.0	8.00	7.40	7.70	7.7
Norfolk sandy loam + 1 per cent of alfalfa meal								
B. subtilis	89.5	88.4	89.0	1.2	2.38	2.26	2.32	5.2
B. subtilis (no. P_2O_5 added)	81.7	79.9	80.8	2.2	2.51	2.51	2.51	0.0
B. megatherium	73.9	71.9	72.9	2.7	1.12	1.37	1.25	20.0
B. megatherium (no. P_2O_5 added)	63.4	60.1	61.8	5.3	1.37	1.24	1.31	10.0
Trichoderma sp.	160.1	146.1	153.1	9.1	0.69	0.57	0.63	*
Trichoderma sp. + Aspergillus niger	167.1	170.2	168.7	1.8	−0.32	−0.07	−0.20	
Aspergillus niger	145.2	138.9	142.1	4.4	−0.32	−0.32	−0.32	
Zygorhynchus Vuil + Trichoderma	133.2	133.1	133.2	0.0	0.44	0.57	0.52	
Zygorhynchus Vuil	104.6	90.5	97.6	14.4	0.95	0.95	0.95	
Soil infusion	253.8	272.4	263.1	7.1	−0.45	−0.45	−0.45	
Average				5.2				9.5

* These ammonia determinations from the fungus cultures were not included because of the small amounts obtained.

A COMPARISON OF THE DUPLICATE DETERMINATIONS OF CARBON-DIOXIDE PRODUCTION AND OF AMMONIA ACCUMULATION

It was observed that the variations between duplicate determinations of carbon dioxide were greater during the first few days. But at the end of 12 days the differences became less. Table 1 gives these duplicate determinations for the organisms discussed in the following pages. Averaging the percentages of error for each set of duplicates it was found that the average error for the carbon dioxide determinations was 5.2 per cent, while that for the ammonia determinations was 9.5 per cent. This indicates that carbon-dioxide production may be fairly accurately measured and that it was more uniform than the ammonia accumulation.

TABLE 2

Carbon dioxide and ammonia produced from 1 per cent of cottonseed meal in Norfolk sandy loam

TIME	B. SUBTILIS		B. VULGATUS		B. MYCOIDES		B. SUBTILIS + B. VULGATUS	
	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3
days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1 and 2	17.7		27.3		13.4		26.9	
3 and 4	37.1	5.30	49.1	none	9.3	0.73	64.8	3.97
5	13.2		25.7		6.2		17.6	
6	13.4	7.53	18.0	2.69	5.6	1.18	32.4	6.88
7 and 8	18.1	8.73	15.9	3.49	5.8	1.88	27.0	9.37
9 and 10	22.8	10.58	23.0	4.86	8.1	1.56	24.6	10.00
11 and 12	14.2	10.89	9.1	8.23	12.0	2.19	15.1	10.69
Totals	136.5		168.1		60.4		208.4	
Per cent of total nitrogen and of total carbon as found	17.8	26.6	21.8	20.1	7.9	5.4	27.1	26.1

THE RELATIVE OXIDIZING AND AMMONIFYING POWER OF SEVERAL PURE CULTURES AND OF MIXTURES OF THE SAME

One per cent of cottonseed meal was the source of organic matter for the series given in table 2. It contained 5.84 per cent of nitrogen and 40.3 per cent of carbon. This was mixed with Norfolk sandy loam to which was added 0.066 per cent of acid phosphate, containing about 16 per cent of water-soluble phosphorus calculated as phosphoric pentoxide. Three-tenths of 1 per cent of precipitated calcium carbonate also was added. These percentages are equivalent to about 2000 pounds and 9000 pounds per acre 9 inches. Table 3 gives data obtained under similar conditions for another series.

Considering first the relative activities of these common soil types, it may be seen that *Bacillus vulgatus* leads in carbon-dioxide production (table 2), oxidizing, in a 12-day period, 21.8 per cent of the carbon added in the cotton-

seed meal. *Bacillus subtilis*, giving considerably less carbon dioxide, caused the accumulation of more ammonia, amounting to 26.6 per cent of the total nitrogen added. A mixture of these two organisms gave a higher carbon-dioxide production than either alone, and an ammonia accumulation approximating that of *B. subtilis*. *Bacillus mycoides*, tested twice (tables 2 and 3), gave very much lower amounts both of carbon dioxide and of ammonia. *Bacillus megatherium* (table 3) was about as active as *B. mycoides* and a mixture of the two gave no increase in carbon dioxide. But mixing *B. vulgatus* with *B. mycoides* caused a very marked increase both in carbon-dioxide production and in ammonia accumulation. This increase in carbon dioxide is easily observed in figure 3. Both figure 2 and figure 3 show that the maximum rate of carbon-dioxide production always occurred before the fifth day of incubation.

TABLE 3

Carbon dioxide and ammonia produced from 1 per cent of cottonseed meal in Norfolk sandy loam

TIME	B. MYCOIDES		B. MEGATHERIUM		B. MEGATHERIUM + B. MYCOIDES		B. VULGATUS + B. MYCOIDES	
	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3
days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1 and 2	12.9		13.8		11.6		36.1	
3	3.0	0.50	3.9	1.19	2.0	0.75	20.6	0.63
4 and 5	5.6	1.45	8.6	1.32	4.8	1.51	43.1	4.81
6, 7, 8 and 9	11.5		9.8		14.8		75.1	
10	2.6		1.7		1.8		8.8	
11 and 12	5.8	1.42	3.7	1.42	4.1	2.57	14.7	7.70
Totals	41.6		41.5		39.1		198.4	
Per cent of carbon and of nitrogen as found	5.4	3.5	5.2	3.5	5.0	6.3	25.8	18.8

A series in which ground alfalfa hay was used instead of cottonseed meal was inoculated with cultures of *B. subtilis* and *B. megatherium* (table 4). As shown in the table, the addition of acid phosphate caused an increase in carbon-dioxide but none in ammonia accumulation. Until more data are secured no conclusions can be drawn in the comparison of carbon-dioxide production in its relation to ammonia accumulation as influenced by phosphates and by other salts.

In a second series, with alfalfa as the source of organic matter, the activities of some fungi were measured and compared with those of a soil infusion (table 5). The average oxidizing power for the bacteria (table 4) was 8.0 per cent, while that for the individual species of fungi was 16.5 per cent of the carbon added. The soil infusions greatly exceeded both, being 33.0 per cent. The bacteria are not able to utilize the carbon of cured alfalfa as readily as fungi,

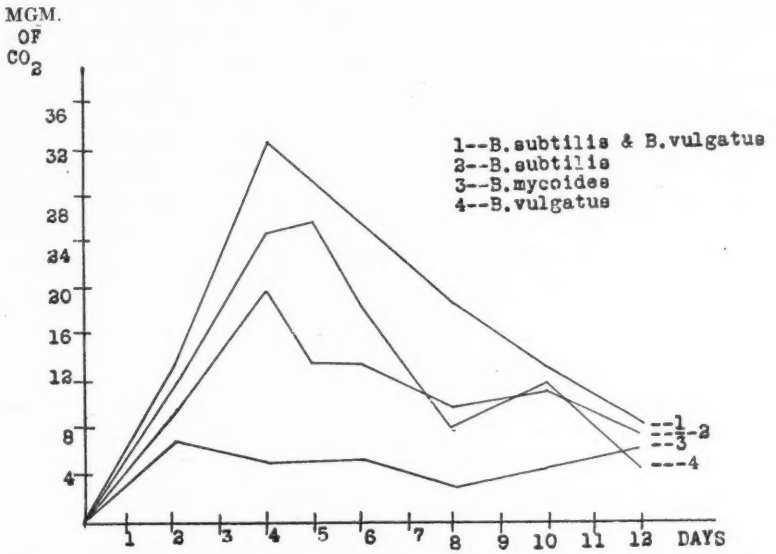


FIG. 2. THE DAILY PRODUCTION OF CARBON DIOXIDE FROM NORFOLK SANDY LOAM, PLUS 1 PER CENT OF COTTONSEED MEAL, BY PURE CULTURES OF BACTERIA (TABLE 2)

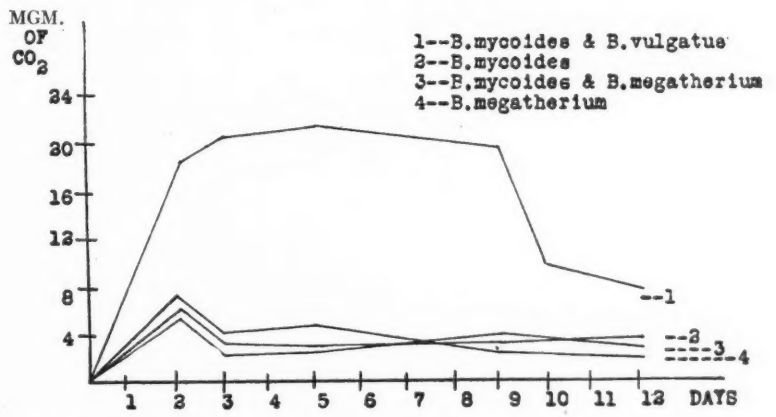


FIG. 3. THE DAILY PRODUCTION OF CARBON DIOXIDE FROM NORFOLK SANDY LOAM, PLUS 1 PER CENT OF COTTONSEED MEAL, BY PURE CULTURES OF BACTERIA (TABLE 3)

and this would be even more evident for a longer period, since the fungus cultures start more slowly but are more active at the end of 12 days.

Glancing again at table 5 it may be seen that when a mixed culture of *Trichoderma* sp. and *Aspergillus niger* is used an activity greater than that of either alone is obtained. *Trichoderma* sp. and *Zygorhychus Vuilleminii* acting together showed an activity less than the one and greater than the other. It would be interesting to know more of the associative action of fungi and bacteria in pure cultures. The combinations of bacterial cultures so far studied, appear to be symbiotic in their behavior. Using ammonia accumulation as an index, Coleman (1) found, for low moisture contents, an antagonistic action between *B. subtilis* and *Zygorhynchus Vuilleminii*. Soil

TABLE 4

Carbon dioxide and ammonia produced from 1 per cent of alfalfa in Norfolk sandy loam with and without the addition of acid phosphate

TIME	B. SUBTILIS				B. MEGATHERIUM			
	Acid phosphate added		No acid phosphate added		Acid phosphate added		No acid phosphate added	
	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3
days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	7.1		5.7		7.8		7.4	
2 and 3	41.6		38.6		30.0		26.6	
4	13.0		8.3		7.4		5.0	
5 and 6	12.6	1.80	11.2	1.63	10.2	1.11	9.8	0.73
7 and 8	6.1		6.6		6.8		4.7	
9, 10, 11 and 12	8.7	2.32	10.1	2.51	10.9	1.25	8.3	1.31
Totals	89.0		80.8		72.9		61.8	
Per cent of carbon and of nitrogen as found	8.4	14.9	6.8	16.1	7.6	8.0	5.8	8.4
Per cent increase due to acid phosphate	10.1				17.9			

infusions are known to be more active than individual organisms. Hence it may be inferred either that the associative action of the many species in an infusion is beneficial or that the most active species have not been isolated, and if they have, that they have been attenuated by artificial culture.

Finally, it may be observed (table 5) that those fungi which oxidized the most gave the lowest accumulations of ammonia. In both cases where *Aspergillus niger* was added, as well as where a soil infusion was employed, the organisms even used up some of the ammonia shown to be present in the sterilized checks (fig. 5).

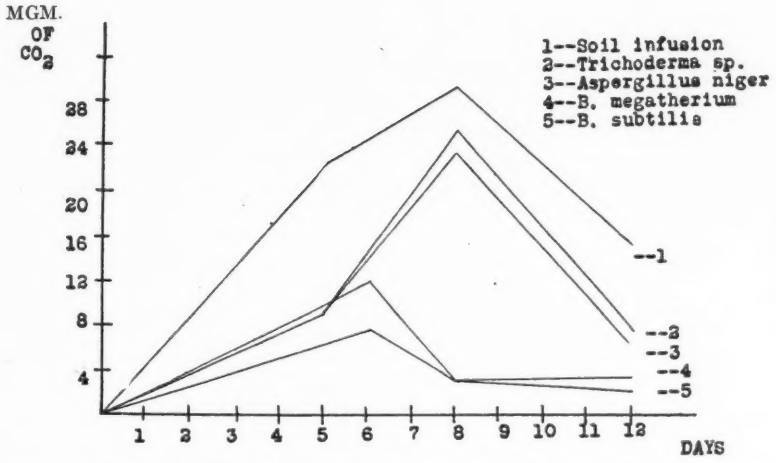


FIG. 4. THE PRODUCTION OF CARBON DIOXIDE BY CULTURES OF BACTERIA AND OF FUNGI AND BY A SOIL INFUSION, WITH 1 PER CENT OF CURED ALFALFA IN NORFOLK SANDY LOAM (TABLES 4 AND 5)

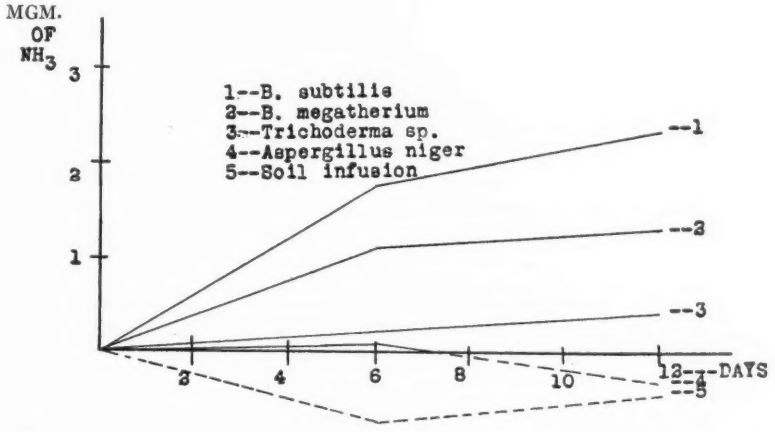


FIG. 5. THE PARALLEL ACCUMULATION OF AMMONIA BY THE ORGANISMS FOR WHICH THE CARBON-DIOXIDE PRODUCTION IS GIVEN IN FIGURE 4

THE CORRELATION BETWEEN CARBON-DIOXIDE PRODUCTION AND AMMONIA ACCUMULATION BY PURE CULTURES OF BACTERIA AND FUNGI AND BY SOIL INFUSIONS

In considering the relation of ammonia accumulation to the rate of organic decay it is of interest to inspect the data so far obtained from parallel determinations of ammonia and carbon-dioxide. Table 6 shows that a soil infusion decomposed nearly three times as much of the alfalfa as *B. subtilis* for a period of 8 days, the percentages of total carbon oxidized being 22.0 and 8.9, respectively. During this period *B. subtilis* caused the accumulation of 10.4 per

TABLE 5

Carbon dioxide and ammonia produced from 1 per cent of alfalfa in Norfolk sandy loam by fungi and by a soil infusion

TIME	TRICHODERMA SP.		ASPERGILLUS NIGER		ZYGORHYNCHUS VUILLEMINII		SOIL INFUSION		TRICHODERMA SP. + ASPERGILLUS NIGER		TRICHODERMA SP. + ZYGORHYNCHUS VUILLEMINII	
	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3
days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1, 2, 3, 4 and 5	46.0	0.04	47.5	0.13	38.0	0.63	112.8	−0.72	45.4	0.45	38.6	0.31
6, 7 and 8	77.6	0.25	70.1	0.00	44.6	0.44	88.3	−0.22	91.0	−0.14	59.8	0.70
9, 10, 11 and 12	29.6	0.63	24.5	−0.32	15.1	0.95	62.1	−0.45	32.3	−0.20	34.9	0.52
Totals.....	153.2		142.1		97.7		263.1		168.7		133.3	
Per cent of carbon and of nitrogen as found.	19.4	4.4	17.7		12.3	6.1	33.0		21.1		16.7	3.4

TABLE 6

The production of carbon dioxide and the accumulation of ammonia from 2 per cent of alfalfa in Norfolk sandy loam by B. subtilis and a soil infusion

ORGANISMS	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH AND 6TH DAYS	7TH AND 8TH DAYS	TOTALS	PER CENT OF CARBON OXIDIZED	PER CENT OF N AMMONIFIED
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
B. subtilis									
CO_2.....	47.3	39.6	16.7	9.8	15.6	11.2	140.2	8.9	
NH_3.....		1.70		2.14	3.13	3.49			10.4
Soil infusion									
CO_2.....	47.0	42.0	68.2	54.6	91.8	41.8	345.4	22.0	
NH_3.....		−0.60		−0.51	−1.20	−1.16			none

cent of the total nitrogen as ammonia, whereas the soil infusion not only caused no accumulation but even used up most of the small amount appearing in the checks after sterilizing.

With alfalfa as the source of energy pure cultures of fungi give results similar to those obtained with a soil infusion (table 5). The less active of the fungi tested gave a small accumulation of ammonia while the more active species, like the soil infusions, used up some of the ammonia originally present in the checks as well as all that may have been liberated.

Looking now at the action of bacteria upon alfalfa it may be noted that they (table 4) cannot utilize it as readily as fungi, the average amount oxidized by the two groups being 8.0 and 17.5 per cent, respectively. But the bacteria, although they oxidized less than half as much carbon, caused the accumulation of 14.2 per cent of the total nitrogen as ammonia, or over three times as much as was accumulated by the fungi, for which the percentage was 4.6, not including the more active fungus cultures which produced no ammonia at all (fig. 4 and 5).

Under the conditions of the experiment, an average of 12.2 per cent of the total carbon of cottonseed meal was oxidized by the bacterial cultures (tables 2 and 3), while the average amount of nitrogen found as ammonia was 13.8 per cent. The action of fungi upon cottonseed meal has not been studied with reference to the simultaneous production of carbon dioxide and accumulation of ammonia. Working with pure cultures, McLean and Wilson (8), and later Coleman (1), Kopeloff (3), and Waksman and Cook (13), have shown that fungi cause considerable accumulation of ammonia from cottonseed meal, the amounts depending upon various factors such as phosphates, length of incubation, moisture, temperature and type of soil. Lipman and Burgess (5), studying several bacteria in pure cultures, found with a given type of soil, that the species giving the highest ammonia accumulation with one kind of organic matter was not always the highest accumulator with another kind.

Referring again to the carbon-dioxide production and the ammonia accumulation from alfalfa, the data so far secured show that a low accumulation of ammonia is an indication of high rather than of low activity. With this type of organic matter the behavior of fungi was more like the action of soil infusions than the behavior of bacteria, indicating that the most active components of a soil flora, as obtained from an infusion, are fungi.

It seems evident that other kinds of organic matter and of soils, as well as the effect of various fertilizing elements and soil amendments, should be studied with reference to the optimum ratio between carbon-dioxide production and ammonia accumulation. The action of pure cultures of bacteria, actinomyces and fungi, when mixed together in different combinations, deserves further consideration and investigation. If these three groups make up the natural soil flora, studies in such combinations or floras made up synthetically might throw more light on the interactions and needs of soil organ-

isms so that they could be better controlled and aided in their function of preparing food for plants.

SUMMARY

The results obtained so far with the use of carbon dioxide as an index of biological activity and the correlation of this activity with ammonia accumulation may be summarized as follows.

1. An apparatus is described for the determination of carbon dioxide evolved biologically from soil.

2. Methods are given which were used for the study of organic decomposition by pure cultures and by mixtures of pure cultures of microorganisms.

3. Duplicate determinations indicate that the production of carbon dioxide is more uniform than the accumulation of ammonia for a 12-day period.

4. In general a high carbon-dioxide production by pure cultures of bacteria was accompanied by a high ammonia accumulation, with 1 per cent of cottonseed meal or of alfalfa in Norfolk sandy loam.

5. Pure cultures of the fungi tested, oxidized more of the carbon of alfalfa than pure cultures of bacteria, but the bacteria caused the accumulation of much more ammonia. The more active species of fungi not only caused no accumulation of ammonia but even used up some of the small amounts appearing in the checks. Soil infusions resembled the fungus cultures with respect to ammonia accumulation but were more active in the production of carbon dioxide.

6. With alfalfa as the source of organic matter a low accumulation of ammonia is an indication of a high rather than of a low activity. Furthermore, since the behavior of the soil infusions was more like that of fungi than of bacteria, it would seem that fungi were the more active components of the natural soil flora.

7. The mixtures of pure cultures of bacteria tested showed no antagonism and in some cases a symbiotic relation seemed to exist. Mixtures of pure cultures of fungi or of fungi and bacteria have not been studied sufficiently to permit of any conclusion therefrom.

In conclusion it is a pleasure to thank Dr. J. G. Lipman for his many helpful suggestions given during the course of this work.

REFERENCES

(1) COLEMAN, D. A. 1916 Environmental factors influencing the activity of soil fungi. *In* Soil Sci., v. 2, no. 1, p. 1–65.

(2) FRED, E. B., AND HART, C. B. 1915 The comparative effect of phosphates and sulfates on soil bacteria. Wis. Agr. Exp. Sta. Research Bul. 35, 32 p.

(3) KOPELOFF, N. 1916 The inoculation and incubation of soil fungi. *In* Soil Sci., v. 1, no. 4, p. 381–403.

(4) LEMMERMANN, O., ASO, K., FISCHER, H., AND FRESENIUS, L. 1911 Untersuchungen über der Zersetzung der Kohlenstofferbindungen verschiedener organischer Substanzen im Boden, speziell unter dem Einfluss von Kalk. *In* Landw. Jahrb., Bd. 41, p. 217–256.

(5) LIPMAN, C. B., AND BURGESS, P. S. 1914 Ammonification in soils by pure cultures. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 7, p. 141–172.

(6) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., AND MCLEAN, H. C. 1912 Experiments on ammonia formation in the presence of carbohydrates and other non-nitrogenous organic matter. N. J. Agr. Exp. Sta. Bul. 247, 22 p.

(7) LIPMAN J. G., AND BLAIR, A. W. 1914 The formation of carbon dioxide and nitrates in the presence of large amounts of carbohydrates. *In* N. J. Agr. Exp. Sta. 35th Ann. Rpt., 1914, p. 220–221.

(8) MCLEAN, H. C., AND WILSON, G. W. 1914 Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270, 39 p.

(9) POTTER R. S., AND SNYDER, R. S. 1916 Carbon dioxide production in soils and nitrogen changes in soils variously treated. Iowa Agr. Exp. Sta. Research Bul. 39, p. 254–309.

(10) STOKLASA, J., AND EARNEST, A. 1905 Über den Ursprung, die Menge und die Bedeutung des Kohlen dioxyds im Boden. *In* Centbl. Bakt. [etc.], abt. 2, Bd. 14, p. 723–736.

(11) TRUOG, E. 1915 Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. *In* Jour. Indus. Engin. Chem., v. 7, no. 12, p. 1045.

(12) VAN SUCHTELEN, F. H. H. 1910 Über die Messung der Lebenstätigheit der äerobiotischen Bakterien im Boden durch die Kohlensäureproduktion. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, p. 45–89.

(13) WAKSMAN, S. A., AND COOK, R. C. 1916 Incubation studies with soil fungi. *In* Soil Sci., v. 1, no. 3, p. 275–284.

(14) WOLLNY, E. 1897. Die Zersetzung der Organischen Stoffe.

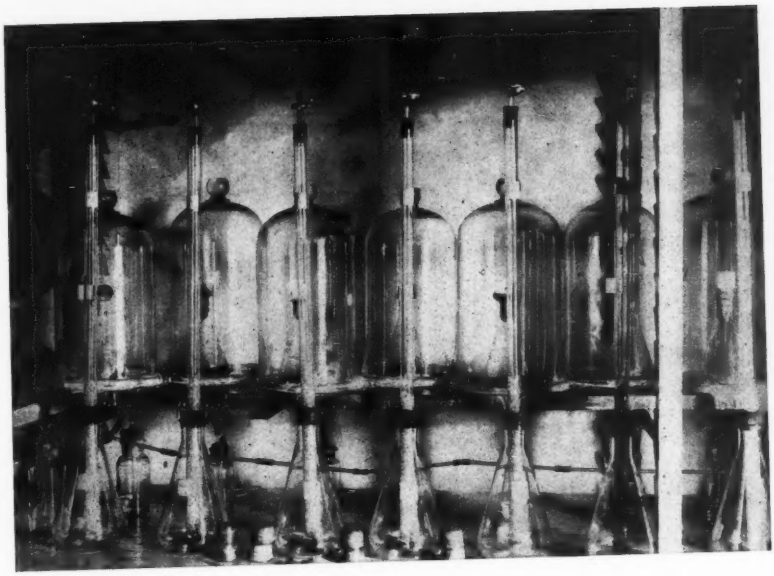
PLATE 1

A part of the carbon-dioxide apparatus set up in the constant temperature room. (Photo by R. F. Poole.)

CARBON-DIOXIDE AND AMMONIA PRODUCTION

J. R. NELLER

PLATE 1



EXPERIMENTS WITH SULFUR-PHOSPHATE COMPOSTS CONDUCTED UNDER FIELD CONDITIONS[1]

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Received for Publication September 28, 1917

SERIES I. COMPOSTS IN EARTHENWARE POTS

It is often advantageous in research work to imitate natural conditions without sacrificing laboratory precision and careful control. Such at least was the need felt in carrying out the sulfur-floats composting experiments. It was desired to have certain mixtures placed in sufficiently direct contact with the soil to create natural conditions of soil moisture and temperature and yet to retain the mixtures well defined, thus assuring representative sampling, and also artificial control of atmospheric moisture. This requirement was met by the use of porous earthenware pots—each provided with a hole in the bottom—placed in slight excavations, which were so adjusted that the tops of the pots were flush with the surface of the soil.

The experiment as already stated was to deal with a variety of conditions rather than with varying combinations of materials.

Four standard mixtures were used, viz.,

No. 1. Red silt loam soil (0.1190 per cent P_2O_5).
No. 2. 2 parts sulfur to 12 parts soil.
No. 3. 6 parts floats to 12 parts soil.
No. 4. 6 parts floats, 2 parts sulfur, and 12 parts soil.

Four sets of these mixtures were made up—the proportions having been so adjusted that there were 20 pounds in each pot. Each set was placed under a different condition. Two sets were covered with waterproof canvas placed on a V-shaped frame open at each end. The other two sets were entirely exposed to the weather. The arrangement of the pots and the treatment which each received were as follows:

[1] See Lipman, J. G., and McLean, H. C. The oxidation of sulfur in soil as a means of increasing the availability of mineral phosphates. *In* Soil Sci., v. 1, p. 533, (1916). Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil Sci., v. 2, p. 499 (1916). Vegetation experiments on the availability of treated phosphates. *In* Soil Sci., v. 4, p. 337 (1917).

1. Soil, compost covered, inoculated.
2. Soil, sulfur, covered, inoculated.
3. Soil, floats, covered, inoculated.
4. Soil, floats and sulfur, covered, inoculated.
5. Soil, uncovered, 0.02 per cent aluminum sulfate, ferrous sulfate, inoculated.
6. Soil, sulfur, uncovered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
7. Soil, floats, uncovered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
8. Soil, floats, sulfur, uncovered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
9. Soil, covered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
10. Soil, sulfur, covered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
11. Soil, floats, covered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
12. Soil, floats, sulfur, covered, 0.02 per cent aluminum sulfate, 0.02 per cent ferrous sulfate, inoculated.
13. Soil, uncovered, inoculated.
14. Soil, sulfur, uncovered, inoculated.
15. Soil, floats, uncovered, inoculated.
16. Soil, floats, sulfur, uncovered, inoculated.

The quantity of available phosphoric acid found in the composts at different intervals is recorded in table 1.

A discussion of the high production of available phosphoric acid where sulfur and floats were combined, as compared with the production in the other composts of each set, scarcely seems necessary. In view of the extensive data recorded elsewhere this was to be expected. Consideration will be given at this time only to the sulfur-floats combinations as affected by the environment to which they were exposed.

Passing over the steady increase of available phosphoric acid at the successive dates of sampling, and comparing the highest results obtained, which is at the end of 20 weeks, we note first of all that the cover protection exerted some influence. The pots under cover exhibit a higher available phosphoric acid yield than do the others. No. 4 shows an accumulation of 36.86 per cent of the total phosphoric acid in an available form, as against 29.68 per cent in No. 16. In the case of the composts which received the aluminum and iron salts we likewise observe a 32.55—32.96 per cent accumulation of available phosphoric acid. As the covered pots were kept under optimum moisture conditions by artificial means, while the uncovered pots were always exposed, it would be expected that a greater chance for leaching would develop there; especially so, since rainy weather prevailed during a considerable part of the first fifteen weeks of the experiment. It is to leaching, therefore, that we ascribe this difference.

The aluminum and iron salts did not, it seems, produce results like those obtained in laboratory experiments. In this instance the ferrous and aluminum sulfates brought about an increase only in the uncovered set, and

this increase is not very large. In compost 8, we find 62.40 pounds of available phosphoric acid, as against 56.20 pounds in compost 16, which received no addition of salts. In the covered set we find 69.80 pounds of available phosphoric acid in the compost receiving no salts as against 61.63 pounds in the treated ones. As the ferrous and aluminum sulfates were not added until 5 weeks after the experiment was started, it is probable that this circumstance might account for the discrepancies noted. In the laboratory experiments the salts were always added when the composts were being started and hence it is probable that a stimulation was exerted at the time when the sulfofiers were developing vigorously.

TABLE 1

The content of available P_2O_5 in the different composts calculated to pounds of P_2O_5 per ton

COMPOST NUMBER	AT BEGINNING	AETER 8 WEEKS	AFTER 11 WEEKS	AFTER 15 WEEKS	AFTER 20 WEEKS	TOTAL P_2O_5 IN 1 TON OF COMPOST	TOTAL P_2O_5 RENDERED AVAILABLE IN 20 WEEKS
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*
1	0.53	0.64	0.63	0.64	0.598	1.95	
2	0.53	0.63	0.64	0.61	0.60	1.67	
3	2.96	2.90	3.32	3.29	3.45	208.76	1.65
4	3.13	8.36	23.51	53.77	69.80	189.32	36.86
5	0.53	0.64	0.61	0.63	0.65		
6	0.53	0.63	0.64	0.58	0.60		
7	2.96	3.24	3.53	3.49	3.80	208.76	1.82
8	3.13	10.82	19.23	49.08	62.40	189.32	32.96
9	0.53	0.58	0.62	0.64	0.65		
10	0.53	0.63	0.64	0.61	0.71		
11	2.96	3.27	3.41	3.29	3.40	208.76	1.62
12	3.13	5.78	14.56	54.04	61.63	189.32	32.55
13	0.53	0.61	0.63	0.62	0.64		
14	0.53	0.63	0.62	0.64	0.78		
15	2.96	3.09	3.49	3.33	3.13	208.76	1.49
16	3.13	8.81	15.91	51.81	56.20	189.32	29.68

SERIES II. PRODUCTION OF AVAILABLE P_2O_5 IN 2-TON COMPOSTS

The theory of available phosphate production from insoluble phosphates, through the medium of sulfur, having been established by laboratory experimentation, the next step would seem to be its commercial utilization.

In the case at hand, the problem contains the practical application under farm conditions rather than from the factory standpoint. How can the individual farmer utilize the sulfur-floats method? This question can be best answered by actual field tests. Accordingly a number of composts of varying composition were made up in the latter part of October, 1916. The soil used was a red silt loam. It was sifted through a quarter-inch mesh sieve to facilitate handling. Tennessee brown rock phosphate containing 31.12

per cent of phosphoric acid, sulfur flour, and composted manure were the materials used in the composts. Samples of the manure and soil were secured and analyzed in order that the per cent of phosphoric acid rendered available in the compost could be calculated. The composts were made up so that at completion each weighed two tons. They were prepared according to the following plan:—

Compost No.

1. 4,000 pounds soil (0.1190 per cent P_2O_5).
2. 3,800 pounds soil, 200 pounds sulfur.
3. 3,600 pounds soil, 400 pounds floats.
4. 3,400 pounds soil, 400 pounds floats, 200 pounds sulfur.
5. 3,600 pounds soil, 400 pounds sulfur,
6. 3,200 pounds soil, 800 pounds floats,
7. 2,800 pounds soil, 800 pounds floats, 400 pounds sulfur.
8. 3,800 pounds soil, 200 pounds manure.
9. 3,600 pounds soil, 200 pounds manure, 200 pounds sulfur.
10. 3,400 pounds soil, 200 pounds manure, 400 pounds floats.
11. 3,200 pounds soil, 200 pounds manure, 400 pounds floats, 200 pounds sulfur.
12. 3,400 pounds soil, 200 pounds manure, 400 pounds sulfur.
13. 3,000 pounds soil, 200 pounds manure, 800 pounds floats.
14. 2,600 pounds soil, 200 pounds manure, 800 pounds floats, 400 pounds sulfur.

In each compost a thorough mixing of the constituents was obtained by shovelling over several times. Moreover, to insure an even mixture the sulfur, where used, was sifted through a fine-mesh sieve before its addition. Fourteen composts were made and exposed to the weather. They were so spaced as to prevent contamination of one heap by another. They were turned over on May 1, 1917. At different intervals samples were secured and analyzed for citrate-soluble phosphoric acid. The dates of sampling, and the results obtained are given in table 2.

The maximum production of available phosphoric acid is shown at the end of 44 weeks. Since 400 pounds of rock phosphate contain 124.48 pounds of phosphoric acid, we at once note the high proportion made available in the composts containing both floats and sulfur. Likewise we note, in contrast, the small amounts of available phosphoric acid obtained where sulfur was not employed with the floats.

We note, further, that the check compost number 1 contained about the lowest amount of available phosphoric acid in its series, numbers 1 to 7; as also did the check compost number 8 for its series, numbers 8 to 14. Sulfur alone and with manure gave no appreciable increase over the check. In the case of the composts 3, 6, 10 and 13, which received floats, but no sulfur, it will be noted that the insoluble phosphates had undergone practically no changes in solubility throughout the entire 44-week period. It is only when the floats are composted with sulfur that a change in availability is effected. The remarkable increase in available phosphoric acid from May 12 to September 3, should be especially noted. It appears that the sulfofication proc-

ess did not gain headway until the advent of warm weather. Once started it apparently developed very rapidly, until, on September 3, we find 96.98 pounds, or 75.87 per cent of the total phosphoric acid, to be available in compost 4 where 400 pounds of floats, 200 pounds of sulfur, and 3400 pounds of soil were composted together. In compost 7 with the quantities of sulfur and floats doubled and the soil reduced to 2800 pounds, we note but a slight increase in available phosphoric acid over that obtained in compost 4. The amount of available phosphoric acid in this case was 99.79 pounds, or 39.65 per cent of the total. The addition of manure did not increase the accumulation of available phosphoric acid in compost 11, for only 96.43 pounds were obtained here. This amount is the same as that obtained in compost 4 which contained no manure. In compost 14, however, the addition of manure evidently favored sulfofication, because 174.38 pounds, or 69.16 per cent of

TABLE 2

The content of available phosphorus in the composts at various intervals calculated to pounds of P_2O_5

COMPOST NUMBER	AT BEGINNING OCTOBER 31, 1916	AFTER 10 WEEKS JANUARY 10, 1917	AFTER 21 WEEKS MARCH 30, 1917	AFTER 27 WEEKS MAY 12, 1917	AFTER 38 WEEKS JULY 23, 1917	AFTER 44 WEEKS SEPTEMBER 3, 1917	TOTAL P_2O_5 IN COMPOST	TOTAL P_2O_5 RENDERED AVAILABLE IN 44 WEEKS
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*
1	0.97	0.96	1.01	0.92	0.95	1.07		
2	0.93	0.95	1.05	1.03	1.02	1.11		
3	3.79	4.25	3.77	4.26	4.89	6.45	127.99	5.04
4	3.85	4.99	5.18	7.88	47.96	96.98	127.82	75.87
5	1.16	1.14	1.14	1.01	1.21	1.16		
6	6.09	4.85	4.85	4.48	6.95	7.51	252.08	2.98
7	5.65	6.50	6.89	8.99	49.99	99.79	251.69	39.65
8	1.27	1.57	1.34	1.36	1.16	2.22		
9	1.41	1.45	1.35	1.38	1.47	1.73		
10	3.36	2.35	3.68	3.33	4.86	5.23	127.80	4.09
11	3.93	4.38	4.83	9.61	56.01	96.43	127.60	75.85
12	1.78	1.50	1.63	1.69	1.61	2.89		
13	4.76	5.46	4.98	4.76	6.95	6.25	252.53	2.47
14	4.25	7.62	7.04	25.58	147.49	174.38	252.14	69.16

the total phosphoric acid, were found to be available in this case as against 99.79 pounds, or 39.65 per cent of the total phosphoric acid, in compost 7, which received the same quantities of sulfur and floats but no manure. On May 12, similar relative results were obtained. From October, 1916, to May 1917, sulfofication was practically suspended, although on May 1 it was observed that where sulfur was one of the constituents of the composts, their exterior, to a depth of one inch, showed an almost total absence of sulfur. This indicated that it had been transformed into some other form, that is, it had been oxidized. Hence it is likely that even during the cold months some action had taken place in the outer layer of the compost, which was influenced either by aeration or temperature.

In conclusion, it appeares that composts should be made up in warm weather, and so constructed as to permit free access of air, if maximum results are to be obtained.

SERIES III. SHALLOW COMPOSTS

Laboratory experiments showed aeration to be an important factor in composting. On May 1, when the composts in the experiment already reported were turned, it was observed that the outer layer to the depth of one inch was devoid of sulfur, indicating that the sulfur was transformed rapidly only in the well aerated outer layer. It was therefore concluded that large composts, especially when placed in large heaps, were not the most desirable. The need of thorough aeration having been indicated, it was decided to prepare composts with a depth of about 15 inches. This was on a small scale, the plots each being only 4 feet square. The soil, a Sassafras gravelly loam, was considered to weigh 80 pounds per cubic foot. With this soil contained

TABLE 3

Available phosphoric acid in shallow compost experiment calculated in pounds per ton of compost

PLOT NUMBER AND TREATMENT	AT BEGINNING	AFTER 6 WEEKS	AFTER 15 WEEKS	AFTER 20 WEEKS
	pounds	*pounds*	*pounds*	*pounds*
1. 320 pounds floats	3.49	3.38	3.89	3.26
2. 320 pounds floats and 106.66 pounds sulfur	3.98	4.96	27.39	35.41

within the 4 x 4 foot plot to a depth of 6 inches, the floats and sulfur were thoroughly mixed. Each compost was inoculated with soil known to possess a high sulfofying power. The composts were stirred every week, thus keeping them well aerated. The plan of the experiment and the results obtained are recorded in table 3.

Each ton of the composted material was calculated to contain 186.72 pounds of phosphoric acid in the floats alone, We therefore note with interest the contrast between the two composts, a low availability with the one; a high availability with the other. At the end of 6 weeks the sulfur-treated floats do not show such contrast, but after that the available phosphoric acid accumulated very rapidly until at the end of 20 weeks we find as much as 35.41 pounds of available phosphoric acid per ton of the air-dry compost as against 3.26 pounds in the compost containing only soil and floats. These results seem to indicate that the better aerated mixtures were more effective than the 2-ton composts, for even at the end of 6 months the latter did not show as great an accumulation of available phosphoric acid as was obtained in this experiment at the end of 15 weeks. One point should be remembered, the 2-ton composts were started in October at the beginning of cold weather, while this experiment was started at the beginning of warm weather (April 18). Nevertheless, this experiment disclosed many advantages existing in

the use of shallow composts, and further trials for this reason were made with composts described in the next series.

SERIES IV. MODIFIED SHALLOW COMPOSTS

This experiment was designed to ascertain the best depth through which sulfur and floats may be distributed in the soil, in order to obtain a maximum accumulation of available phosphoric acid. Five small plots, 2 feet square, were used. The soil within each area was removed to a different depth for each plot. The depths were; ½ inch, 1 inch, 2 inches, 3 inches, and 4 inches. The soils from the different plots were mixed together and the following proportionate mixture made:

100.00 parts Tennessee brown rock phosphate.
33.33 parts sulfur flour.
200.00 parts soil.

The plot excavations were filled with this mixture flush with the surrounding soil. Each plot received 0.02 pound of ferrous sulfate and 0.02 pound of aluminum sulfate for each 100 pounds of the compost mixture. In each case

TABLE 4

Influence of depth on the accumulation of available phosphoric acid: Available P_2O_5 calculated in pounds per ton of compost

PLOT NUMBER	DEPTH	AVAILABLE P_2O_5		
		At beginning	After 7 weeks	After 14 weeks
	inches	*pounds*	*pounds*	*pounds*
1	½	3.97	9.60	13.40
2	1	3.97	9.17	16.20
3	2	3.97	9.95	21.20
4	3	3.97	15.37	24.95
5	4	3.97	16.00	25.90

inoculation was made with soil known to be high in its sulfofying power. Cultivation was made easy by the flatness of the composts. This was the principal motive for making them flush with the ground. The composts in the preceding experiments were heaped up to a certain degree. The flat compost would represent the farmer's possibility of making available phosphorus by the sulfur-floats method in the open field. Under such conditions the soil could be stirred by ordinary tillage implements, and this would constitute the most economical method of securing adequate aeration. In this experiment the plots were cultivated once a week. Samples were taken at the beginning and moisture and available phosphorus determined. At the end of 7 and 14 weeks, respectively, samples were also secured and analyzed. The plan of the experiment and the data secured are recorded in table 4.

At the end of 7 weeks we note not only a decided gain of available phosphorus over the amount present at the beginning, but also observe comparatively wide differences between the composts themselves. The depths of ½, 1 and 2 inches are nearly identical, each containing between nine and ten pounds of available phosphoric acid. The depths of 3 and 4 inches are also identical but contain considerably more available phosphoric acid, the amount being 15.37 and 16.00 pounds of P_2O_5, respectively. As the months of June and July were somewhat rainy it is very probable that considerable leaching occurred in the very shallow composts. The relatively higher results obtained at the end of 14 weeks would substantiate this. Nevertheless, the depths of 3 and 4 inches still show the largest amount of available phosphoric acid. The large amounts of 24.95 and 25.90 pounds of P_2O_5 in such a short period of time are very gratifying, making it appear that this method of composting will prove most acceptable for practical use by the individual farmer.

SUMMARY

1. The results obtained by the authors in experiments carried on under field conditions show that the sulfur-floats-soil compost may be utilized in rendering available the phosphorus of floats, thus confirming earlier experiments reported from the laboratories of the New Jersey Agricultural Experiment Station.

2. Experiments are reported which were designed to determine the best form of compost from the standpoint of adequate aeration.

3. Temperature is an important factor in sulfur oxidation. The results obtained would indicate that for maximum efficiency composts should be made up in warm weather.